National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

UNIVERSITY OF ALBERTA

DEVELOPMENTAL VARIABILITY AND THE INDUCTION OF METAMORPHOSIS IN *HAMINAEA CALLIDEGENITA* (MOLLUSCA: OPISTHOBRANCHIA).

BY

Glenys Dianne Gibson ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

DEPARTMENT OF ZOOLOGY

Edmonton, Alberta

Fall 1993

National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-88084-8

Canada

UNIVERSITY OF ALBERTA
RELEASE FORM

NAME OF AUTHOR: Glenys Dianne Gibson

TITLE OF THESIS: Developmental variability and induction of metamorphosis in *Haminaea callidegenita* (Mollusca: Opisthobranchia).

DEGREE: Doctor of Philosophy

YEAR THIS DEGREE GRANTED: 1993

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

Glenys Gibson

PERMANENT ADDRESS:
P. O. Box 35, Canning,
Nova Scotia, Canada.
B0P 1H0

DATED: 26 August 1993

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled DEVELOPMENTAL VARIABILITY AND THE INDUCTION OF METAMORPHOSIS IN *HAMINAEA CALLIDEGENITA* (MOLLUSCA: OPISTHOBRANCHIA) submitted by Glenys Dianne Gibson in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dr. F. S. Chia, Supervisor

Dr. J. C. Holmes

Dr. A. N. Spencer

Dr. P. Fankboner

Dr. A. R. Palmer, Chair

Dr. A. Kohn, External Examiner

Date: 26 August 1993

ABSTRACT

Haminaea callidegenita (Mollusca: Opisthobranchia) has variable development: both swimming larvae and non-swimming juveniles are released from each egg mass. This life history mode is advantageous because every individual produces both dispersive offspring with the potential for colonization of new habitats and gene flow among existing populations, as well as juveniles that immediately recruit to the parental population, resulting in rapid population growth. True variable development is unusual making these rare species invaluable in the study of life history evolution because they allow examination of basic questions without the complications arising from multiple species comparisons.

Variable development in *Haminaea callidegenita* arises from two factors: time of metamorphosis and presence of a metamorphic inducer. Approximately half of the larvae metamorphose inside the egg mass and hatch as juveniles (mean of 60 %, ranging from 4-100 %); the remaining larvae metamorphose within 2 weeks of hatching. Intracapsular metamorphosis is induced by a small (<1000 Da), polar compound that is located in the egg mass jelly. Time of metamorphosis varies both within and among clutches. Metamorphosis within one clutch occurs throughout the pre- and post-hatching periods (4-5 d, and 14-30 d respectively). Metamorphosis is initially rapid and then continues at a low rate throughout the planktonic period. This results in a rapid decrease in the number of dispersers with length of the planktonic period; therefore, the benefits of short range dispersal (e.g., gene flow, colonization) may outweigh the risks associated with a long larval period (e.g., predation, advection from favorable habitats). Larvae gradually respond to an increasing number of natural inducers within this period, increasing the probability of larval contact with an acceptable habitat.

Time of intracapsular metamorphosis is highly heritable ($h^2 = 0.76$) suggesting that it may respond rapidly to selection. It is also genetically correlated with time of metamorphosis immediately after hatching; therefore, if selection increased the proportion of juveniles released, the length of the planktonic period would increase as well and variable development would be maintained. Correlations among other life history traits were also examined. Most appeared phenotypically to be independent; however, they did correlate genetically and environmentally indicating that these traits have not evolved independently.

ACKNOWLEDGEMENTS

I wish to thank Dr. Fu-Shiang Chia for supervising this thesis. My thesis has benefited not only from his insight but also from his unfailing support which has made my graduate student life enjoyable. I also thank Drs. J. C. Holmes and A. N. Spencer for their advice, comments and criticisms as members of my thesis committee. Dr. A. O. D. Willows kindly allowed my use of Friday Harbor Labs, University of Washington, throughout most of the tenure of this thesis. I also thank the library at Friday Harbor labs for the use of their excellent facility.

Two people have given me considerable advice and time in regards to this research. Dr. R. Andersen (Department of Oceanography, University of British Columbia) guided my attempt to identify the metamorphic inducer in *Haminaea callidegenita*. I appreciate his kind permission to use his laboratory facilities and primarily am grateful for his time and that of others in his lab (Dave, Judy and Ianna) in attempting to identify the inducer with NMR. I am also grateful to Dr. F. Yeh (Department of Forest Sciences, University of Alberta) for guiding me through the genetic analyses in chapters 5 and 6. Those chapters have greatly benefited from his patient assistance.

Several organizations have provided me with access to collection sites. Doug Bulthius, Research Coordinator of Padilla Bay National Estuarine Research Reserve allowed me access to my primary field site. Bill Dewey kindly gave me access to the Samish Bay Oyster Company, the original collection site for *Haminaea callidegenita*. Also, Washington State Parks allowed me to collect at Spencer Spit.

This thesis benefits from the advice and criticisms of several readers: Mary Sewell, Steve Adolf, Cam Goater, Sarah Cohen, and Amy Sewell. Special thanks go to Dianna Padilla for reminding me to stick to "just the facts, M'am". One particular reader (M. A. Gibson) actually read the whole thing. This thesis also benefits from conversations with Megumi and Richard Strathmann. Rob Toonen provided invaluable assistance and counting expertise. I greatly appreciate the support, input and particularly the friendship of Sandra Zohar, Jon. Havenhand, Joe Pawlik and Ron Koss. Mark and Tracy McClintock allowed me to use their facilities for final thesis production. Terry Taerum of University of Alberta Computer Services guided my adventures with MTS. T. S. Elliott provided me with many enjoyable hours of Quantz, Stamitz, and Loellielet.

I also thank my father M. A. Gibson for instilling in me an enjoyment of natural history and a curiosity for how things live. This has created in me an appreciation for biology as an avocation as well as research as a career. Thanks also to John P. Donahue for his support, encouragement, and for making everything just so.

TABLE OF CONTENTS

PAGE

Chapter 1. General introduction

Life histories of benthic marine invertebrates 1
Poecilogony 2
Natural history of the opisthobranch *Haminaea callidegenita* 3
Objectives 4
Organization of thesis 5
Literature cited 7

Chapter 2. Poecilogony in the opisthobranch mollusc *Haminaea callidegenita.*

Introduction 11
Materials and methods
Animal collection and culture 13
Experimental Procedures
Reproductive traits 14
Egg mass size 14
Effects of culture conditions 14
Role of egg mass jelly as a metamorphic inducer 15
Results
Description of poecilogony in *Haminaea callidegenita* 16
Factors influencing the proportion of juvenile hatchlings
Reproductive traits 16
Egg mass size 17
Effects of culture conditions 17
Effects of source of inducer vs embryos 18
Discussion 18
Conclusion 20
Literature cited 27

Chapter 3. Metamorphosis in the opisthobranch *Haminaea callidegenita*: competence, delay and the effects of metamorphic inducers.

Introduction 29
Materials and methods
Animal collection and culture 30

Chapter 3. con't.

Time of metamorphosis throughout the larval period

Pre-hatching metamorphosis..... 31

Post-hatching metamorphosis..... 32

Variable induction among clutches..... 32

Results

Time of metamorphosis throughout the larval period

Pre-hatching metamorphosis..... 32

Extracapsular metamorphosis 33

Time of competence throughout the metamorphic period 33

Variable induction of metamorphosis among clutches 34

Discussion

Length of the larval period and time of metamorphic competence ... 35

Metamorphosis inducing activity of different substrates 37

Delay of metamorphosis 38

Conclusion 38

Literature cited 46

Chapter 4. Partial purification of metamorphic inducer from egg mass jelly in the opisthobranch *Haminaea callidegenita.*

Introduction 50

Materials and methods

Larval culture 51

Larval assays 51

Characterization of the metamorphic inducer

General stability..... 52

Effects of proteases..... 52

Molecular weight 53

Extraction and purification

Organic solvents 54

HPLC 54

Activity of the metamorphic inducer

Activity as a factor of egg mass jelly age 55

Presence of inducerin jelly produced by other species 55

Inductive effects of jelly on larvae of other species 56

Chapter 4: con't
Results
Induction of metamorphosis by egg mass jelly 57
Isolation of the metamorphic inducer 57
Purification of the metamorphic inducer (HPLC) 57
Activity of the metamorphic inducer 58
Discussion
Purification of the metamorphic inducer 59
Biological characteristics of the metamorphic inducer 60
Literature cited..... 72

Chapter 5. Dispersal polymorphism in *Haminaea callidegenita* larvae (Mollusca: Opisthobranchia) originates from genetic and environmental factors.
Introduction 74
Materials and methods
Animal culture 75
Genetic analysis
Breeding design and definition of traits 76
Statistical analysis 79
Parental environment 80
Comparison among populations 81
Results
Life history traits
Description of individual traits 81
Influence of female size 82
Seasonal variation 82
Egg size 83
Estimates of heritability 83
Time of metamorphosis
Description of individual traits 84
Estimates of heritability 84
Effects of parental environment 85
Inter-population differences 86
Discussion 86
Reproductive traits 86
Time of intracapsular metamorphosis..... 88

Chapter 5: con't
Metamorphosis after hatching 89
Conclusion 90
Literature cited 106

Chapter 6. Phenotypic and genetic correlation between life history traits and time of metamorphosis in the mollusc *Haminaea callidegenita*.
Introduction 109
Materials and methods
Animal culture and breeding design 110
Statistical and genetic analysis
Statistical model 111
Analysis of individual traits 112
Correlations among traits 112
Results
Correlations among life history traits and metamorphosis 114
Time of metamorphosis and ability to delay metamorphosis
Correlations with reproductive traits 116
Time of metamorphic competence 117
Ability to delay metamorphosis 117
Correlations between competence and delayed metamorphosis.. 118
Discussion 118
Conclusion 121
Literature cited..... 129

Chapter 7. Poecilogony in *Haminaea callidegenita* : General conclusion 131
Literature cited 134

LIST OF TABLES

TABLE		PAGE
2.1	Summary of reproductive traits in *Haminaea callidegenita*	21
2.2	Effects of reproductive traits on intracapsular metamorphosis	22
2.3	Effects of egg mass size on intracapsular metamorphosis	23
2.4	Effects of embryo *vs.* inducer source on intracapsular metamorphosis	23
3.1	Induction of metamorphosis before hatching	40
3.2	Extracapsular metamorphosis in response to various substrates	41
3.3	Variable induction of metamorphosis among clutches	42
5.1	Definition of life history traits examined in *Haminaea callidegenita*	91
5.2	Summary of mean values of life history traits in *Haminaea callidegenita*	92
5.3	Effects of female size on reproductive traits and time of metamorphosis	93
5.4	Effects of spawn time on reproductive traits and metamorphosis	94
5.5	Egg size and effects on time of metamorphosis	95
5.6	Heritability estimates for reproductive traits	96
5.7	Time of metamorphosis after hatching	97
5.8	Heritability estimates for time of metamorphosis	98
5.9	Effects of parental environment on time of metamorphosis	99
5.10	Inter-site differences in the proportion of juvenile hatchlings	100
6.1	Means and heritabilities of life history traits	122
6.2	Correlations among life history traits	123
6.3	Correlations among life history traits and time of metamorphosis	125

LIST OF FIGURES

FIGURE		PAGE
2.1	Variability in the proportion of juveniles hatching from *Haminaea callidegenita* egg masses	24
2.2	Effect of spawning interval on the proportion of juvenile hatchlings	25
2.3	Effect of culture conditions on the proportion of juvenile hatchlings	26
3.1	Intracapsular metamorphosis in response to various substrates	43
3.2	Cumulative % metamorphosis throughout metamorphic period	44
3.3	Rates of metamorphosis (%/ d) throughout the metamorphic period	45
4.1	General stability of metamorphic inducer in egg mass jelly	62
4.2	Effects of proteases on inducer activity	63
4.3	Estimation of inducer molecular weight	64
4.4	Extraction of inducer with organic solvents	65
4.5	Purification of inducer with HPLC (buffer gradient)	66
4.6	Re-partitioning inducer with HPLC (buffer gradient)	67
4.7	Purification of inducer with HPLC (isocratic gradient)	68
4.8	Effect of egg mass jelly age on inductive activity	69
4.9	Inductive activity of egg mass jelly of other species	70
4.10	Inductive activity of egg mass jelly on the larvae of other molluscs	71
5.1	Location of collection sites	101
5.2	Outline of the hierarchical breeding design used for sib analysis	102
5.3	Effect of female weight on hatching period and on egg mass size	103
5.4	Effects of parental environment on the proportion of juvenile hatchlings	104
5.5	Inter-site comparison of proportion of juvenile hatchlings	105
6.1	Correlations among life history traits	126
6.2	Correlations for time of metamorphosis	127
6.3	Correlations for competence and ability to delay metamorphosis	128

Chapter 1

General Introduction

Life histories of benthic marine invertebrates

Benthic marine invertebrates have incredibly diverse and complex life histories. The basic pattern is of a benthic, sedentary adult producing a planktonic larva that, after an obligatory period in the water column, must settle to a specific benthic habitat before metamorphosing into a juvenile (Thorson 1950). Larval type is the basis for the classification of invertebrate life histories and includes: planktotrophy, including species that produce large numbers of tiny feeding larvae that develop during a long planktonic period (weeks to months); lecithotrophy, including species that produce fewer, larger larvae that are non-feeding and have a shorter planktonic period (hours to days); and direct development, including species in which the entire larval period takes place within a benthic egg mass or capsule, and metamorphosis is either complete, vestigial, or lost (ametamorphic development; Bonar 1978) (Thorson 1950; Chia 1974; Day & McEdward 1984; Grahame & Branch 1985). Most species have planktonic larvae (80%; Thorson 1950); of these, the majority are planktotrophic (Jablonski & Lutz 1983).

Larvae are not only extremely variable among taxa but also are vastly different from their adult counterparts, in terms of their morphology, ecology and behaviour. This diversity has made larval type the focal point in theoretical considerations of life history evolution in marine invertebrates (Stearns 1976, 1977; Caswell 1981, 1982; Roughgarden et al. 1985, Roughgarden 1989). The role of the planktonic larva has been extensively discussed in terms of dispersal, gene-flow, speciation, predation, adult energetics, and phylogeny (Day & McEdward 1984; Grahame & Branch 1985; Strathmann 1986; also see Jablonski & Lutz 1983).

Theories of life history evolution are based on intraspecific variation (e.g., egg size: Lessios 1987; McEdward & Chia 1991; Qian & Chia 1991) and comparisons among related species (e.g., Jackson & Strathmann 1981; Hadfield & Miller 1987). These approaches have provided much information, but also have certain drawbacks. Intraspecific studies lack the range in expression of traits that may be required for some comparisons, while inter-specific comparisons risk emphasizing differences in morphology, physiology, and behaviour rather than differences in life history mode. These problems can be avoided by analyses of life history traits in species with poecilogonous, or variable, development. Such species are rare among benthic marine invertebrates, but a consideration of their life histories should provide valuable comparative data.

Poecilogony

Poecilogony is variable development within one species. It was first defined by Giard (1905) who listed dozens of examples in which adults of one species produced different kinds of offspring either seasonally, among different populations, or in response to different environments. He suggested that the larvae diverged by adapting to different environments, while the similarity of adults was maintained by heredity (Giard 1905). Such developmental flexibility has attracted considerable interest because benthic marine invertebrates in general exhibit only one life history mode per species (Thorson 1950). This is so widely accepted that developmental pattern is often used as a taxonomic character. The advantage of poecilogony is that a species obtains the benefits of more than one life history pattern; for example, by producing both planktonic (indirect developing) and juveniles (direct developing) offspring. Planktonic larvae provide the primary dispersive stage for most species of benthic marine invertebrate (Thorson 1950). Therefore, a poecilogonous species would have the advantage of both the dispersal capability required to establish new populations, as well as the ability to supply new recruits to rapidly expand the existing or newly founded population (Blake & Kudenov 1981).

Unfortunately, most examples of poecilogony have been disproven on the grounds that descriptions are based on cryptic species or laboratory artifacts (reviewed by Hoagland & Robertson 1988; in gastropods, Bouchet 1989). A few cases of poecilogony have not been refuted but may still be subject to doubt for two reasons: 1) most observations involve separate individuals and therefore may involve cryptic species; and 2) observations are limited to opisthobranch molluscs and polychaete annelids, both of which are often difficult to assess taxonomically (Hoagland & Robertson 1988).

Perhaps the best studied example of poecilogony is the polychaete *Streblospio benedicti* in which different individuals produce either planktotrophic (feeding) or lecithotrophic (non-feeding) larvae (Levin 1984). Hybridization results in viable offspring (Levin 1984; Levin & Huggett 1990) and additive genetic variance determines the length of the larval period (1-6 weeks for planktotrophs, 0-1 week for lecithotrophs), although was not detected for larval feeding (Levin et al. 1992). The opisthobranch *Elysia chlorotica* also has development that varies between populations, one with planktotrophic and one with direct development. Individuals from these two populations were successfully hybridized producing an F2 generation that was intermediate in larval size and duration of the swimming phase (West et al. 1984). However, both of these cases of poecilogony have been subject to doubt by other authors (e.g., Hoagland & Robertson 1988) because poecilogony has not been observed in one individual. Other proposed cases of

poecilogony lack the support of experimental evidence demonstrating that only one species is involved and that observations do not reflect artifacts (Hoagland & Robertson 1988).

Poecilogony continues to attract attention despite this controversy partly because it is interesting in terms of evolution, but also because it seems likely that it should occur. Benthic marine invertebrates have life history patterns that are diverse and highly variable. Given the diversity observed among taxa and intraspecific variability of traits within any one species, it seems likely that some species should show characteristics of more than one developmental mode during speciation events (Hoagland & Robertson 1988) and perhaps even when evolutionarily stable.

The primary objective of this thesis is to examine poecilogony in the mollusc *Haminaea callidegenita* (Opisthobranchia: Cephalaspidea; Gibson & Chia 1989a). *H. callidegenita* is highly unusual in that both veligers and juveniles are released from every egg mass, with a mean of 60 % juveniles/ egg mass (this study). Both types of offspring are produced by each individual (Gibson & Chia 1989b), thereby eliminating questions regarding taxonomy. Both types of hatchling pass through the same developmental stages and both hatch simultaneously. Intracapsular metamorphosis (occurring within the egg mass) is induced by a compound found in the egg mass jelly (Gibson & Chia 1989b). Hatched veligers may metamorphose 3-4 d after hatching, or may remain swimming for up to 30 d (this study). The length of the planktonic period suggests that poecilogony in this species does not represent a laboratory artifact in which a few larvae escape prematurely from what otherwise would be an egg mass with direct development. The advantage of this life history pattern is that every individual will simultaneously produce both dispersive propagules as well as juveniles that immediately recruit into the parental population.

Natural history of the opisthobranch *Haminaea callidegenita*.

Haminaea are shelled opisthobranchs that are found worldwide, most often intertidally in muddy bays and seagrass beds. Since the genus was introduced by Leach (in Gray 1847), approximately 120 species have been described. Descriptions were based primarily on the shell and contain little information on either adult morphology or natural history. Adults are oblong with a broad head, posterior cephalic shield, and two lateral lobes that nearly cover the shell (Leach, in Gray 1847). Adults are generally 20-30 mm in length (maximum 70 mm in *H. navicula*: Thompson & Brown 1976) and are often brown in colour, although a few tropical species are brightly coloured (Heller & Thompson 1983; Kay 1979). They are simultaneous hermaphrodites with unilateral copulation involving the transfer of spermatophores (Beeman 1977). In the few species studied, *Haminaea* have an annual life cycle with planktotrophic (Richards 1921; Harrigan & Alkon 1984; Gibson &

Chia 1989c) or lecithotrophic (Berrill 1931; Usuki 1966) development. Anecdotal notes on diet indicate that most species graze epiphytes (MacNae 1962; Burn 1966), are herbivorous (Rudman 1971; Powell 1979), or ingest small bivalves (MacPherson & Gabriel 1962; Thompson & Brown 1976). They move by ciliary action through extensive mucous tubes (Rudman 1971) which create polar "trails" that are often followed by other adults (pers. obs.)

Haminaea callidegenita (Gibson & Chia 1989a) is found Washington State, U. S. A. . Adults are approximately 30 mm in length, are reddish-brown in colour and are distinguished from the locally common *H. vesicula* by a deeply bifurcate cephalic shield. They are found in intertidal seagrass beds in bays with slightly warmer water than in nearby channels (pers. obs.). They graze epiphytic diatoms and detritus from seagrass blades (*Zostera marina* and *Z. japonica*), sea lettuce (*Ulva* sp.) and the filamentous green alga *Chaetamorpha linum*, or from surface sediments when seagrass is unavailable during seasonal die-back. Spawning occurs from spring to late fall in the field, although it may continue year-round in mild winters. In severe winters, *H. callidegenita* remain as juveniles in surface sediments and spawning resumes as animals mature in the spring. Recruitment occurs throughout the reproductive season (Gibson & Chia 1991).

Objectives

In this thesis, I described poecilogony in the opisthobranch *Haminaea callidegenita*. *Haminaea* meets two of the basic criteria thought necessary to demonstrate poecilogony as a life history mode. First, poecilogony occurred in the offspring of every individual and therefore eliminates the possibility that this case of variable development reflects sibling species. Second, poecilogony occurred in every egg mass observed and therefore occurred throughout the reference population. This indicates its potential ecological importance to this species as well as providing evidence that it is not a laboratory artifact.

The second major objective was to determine what factors influence developmental variability in this species by affecting the percentage of juveniles (those undergoing intracapsular metamorphosis) hatching from each egg mass. The following questions were addressed:

1. Is poecilogony influenced by physical egg mass characteristics (e.g., size, number of embryos), development time (duration of the embryonic and larval periods), or "female" reproductive traits (size, spawning season)?

2. Is the variance in intracapsular metamorphosis observed among egg masses (ranging from 4-100 %; this study) genetic? Is it correlated with other life history traits?

3. Is the percent intracapsular metamorphosis/ egg mass altered by environmental or culture conditions?

4. What is the inducer that causes intracapsular metamorphosis?

5. Why do only half of the veligers per egg mass respond to the metamorphic inducer before hatching, and what triggers metamorphosis in these larvae after hatching?

Poecilogony is a rare and controversial life history mode in benthic marine invertebrates. *Haminaea callidegenita* is an unusual species in which to study poecilogony because it is the only species known in which a single individual will consistently produce two kinds of offspring. Because of this unusual pattern, it is possible to examine poecilogony itself, as well as to address aspects of variable development that have more general implications for other species with a single mode of development, such as the time of metamorphosis and identification of the metamorphic inducer.

Organization of thesis

This thesis included 7 chapters, each addressing a set of parameters that may influence poecilogony, or addressing a consequence of poecilogony that may have broader implications for other taxa. These chapters inevitably overlapped in terms of methods and pertinent literature, but I have made an effort to limit such repetitions.

Chapter 1 introduced poecilogony and listed the objectives of this thesis.

In Chapter 2, I described poecilogony in *Haminaea callidegenita* and discussed factors that may influence developmental variability including egg mass characteristics, female reproductive traits and the role of the metamorphic inducer. Results indicated that the percent intracapsular metamorphosis varied among larvae of different clutches and was independent of most extrinsic sources.

Chapter 3 addressed time of metamorphosis in *Haminaea* veligers. Time of metamorphosis determined the percentage of juveniles (larvae that metamorphose within the egg mass) and veligers (metamorphose up to 30 d after hatching) hatching from each egg mass. Metamorphosis was examined as the time that metamorphic competence occurs in veligers of one clutch, the ability of hatched veligers to delay metamorphosis, and the changing roles of various natural substrates that induce metamorphosis throughout the planktonic period. I followed all larvae through metamorphosis with the assumption that variable time of metamorphosis throughout the larval period was a viable aspect of this life history mode and veligers that were late "bloomers" did not represent physiologically "inferior" offspring.

In Chapter 4, I examined the chemical cue that induces intracapsular metamorphosis, and therefore poecilogony, in this species. The metamorphic inducer was

found in egg mass jelly (Gibson & Chia 1989b) and I described techniques used to extract and purify the inducer from the jelly matrix. In addition to describing the chemical characteristics of the inducer, I addressed biological features of the jelly that may influence its activity. The inducer was a small, polar compound that was stable over a variety of conditions. It was found in the egg masses of other species but its activity was specific to *Haminaea callidegenita*.

Chapter 5 examined the genetic basis for poecilogony using a hierarchical design allowing both full and half-sib analysis. Poecilogony occurred because larvae become competent to metamorphose throughout both the pre- and post hatching periods; this determined the proportion of veligers or juveniles hatching from each egg mass. I estimated heritability for time of competence, ability of larvae to delay metamorphosis, and other life history traits. The potential effect of parental environment was also examined. Included in this chapter were details on the reproductive biology of *H. callidegenita* throughout the spawning season. Although incomplete reproductive data is available for many opisthobranchs, few studies have provided comprehensive studies throughout the spawning season (Todd 1979; Hall & Todd 1986; Todd & Havenhand 1988); therefore, these data may be useful to other authors because of their completeness and also because they report year comparisons between two laboratory populations.

In Chapter 6, I continued the sib analysis to include genetic (additive and non-additive) and environmental correlations among life history traits. These correlations were compared with the ultimate phenotypic expressions of the various traits considered. Traits included reproductive traits of the females, time of metamorphic competence and ability to delay metamorphosis of offspring. There are two major contributions of this analysis. First, many traits appear phenotypically to be independent, but genetic and environmental comparisons reveal that they are highly correlated. Second, although the evolution of life history patterns in benthic marine invertebrates has been much discussed, to date there is only one other study (Lévin et al. 1992) in which the genetics of life history traits have been considered. The phenotypic data supplied in other studies is valuable, but it is difficult to understand the role of these traits in life history evolution without an understanding of the underlying genetic mechanisms (Falconer 1989).

Chapter 7 was a general discussion of poecilogony in *Haminaea callidegenita*.

Literature Cited

Beeman, R. D. 1977. Gastropoda: Opisthobranchia. *In* Reproduction of Marine Invertebrates, Vol. 4. Ed.: A. C. Giese and J. S. Pearse. London: Academic Press. pp. 115-179.

Berrill, N. J. 1931. The natural history of *Bulla hydatis* Linn. *J. mar. biol. Assoc. U. K.* **17**:567-571.

Blake, J. A. and J. D. Kudenov. 1981. Larval development, larval nutrition, and growth for two *Boccardia* species (Polychaeta: Spionidae) from Victoria, Australia. *Mar. Ecol.Prog. Ser.* **6**(2):175-182.

Bonar, D. 1978. Morphogenesis at metamorphosis in opisthobranch molluscs. *In* Settlement of Marine Invertebrate Larvae. *Ed.*: F. S. Chia and M. E. Rice. New York: Elsevier. pp. 177-196.

Bouchet, P. 1989. A review of poecilogony in gastropods. *J. Moll. Stud.* **55**:67-78.

Burn, R. 1966. Notes on some opisthobranchs mainly from South Australia. *Rec. South Aust. Mus.* **15**(2): 330,331,335.

Caswell, H. 1981. The evolution of "mixed" life histories in marine invertebrates and elsewhere. *Am. Nat.* **117**(4):529-536.

_____. 1982. Optimal life histories and the maximization of reproductive value: a general theorem for complex life cycles. *Ecology*: **63**:1218-1222.

Chia, F. S. 1974. Classification and adaptive significance of developmental patterns in marine invertebrates. *Thalassia Jugosl.* **10**:121-130.

Day, R. and L. McEdward. 1984. Aspects of the physiology and ecology of pelagic larvae of benthic marine invertebrates. *In*: K. A. Steidinger and L. M. Walker (*eds.*) Marine Plankton Life Cycle Strategies. Boca Raton, FL: CRC Press. pp. 93-120.

Falconer, D. S. 1989. Introduction to Quantitative Genetics, 3rd Ed. New York: Longman Press.

Giard, A. 1905. La Poecilogonie. *Comp. Rend. Six. Congr. International de Zool, Berne*, 1904: 617-646.

Gibson, G. D. and F. S. Chia. 1989a. Descripion of a new species of *Haminoea, H. callidegenita* (Mollusca: Opisthobranchia), with a comparison with two other *Haminoea* species found in the northeast Pacific. Can. J. Zool. **67**:914-922.

_____. 1989b. Developmental variability (pelagic and benthic) in *Haminoea callidegenita* (Opisthobranchia: Cephalaspidea) is influenced by egg mass jelly. *Biol. Bull. (Woods Hole, Mass)* **176**:103-110.

_____. 1989c. Embryology and larval development of *Haminaea vesicula* Gould (Opisthobranchia: Cephalaspidea). *Veliger* **32**:409-412.

_____. 1991. Contrasting reproductive modes in two sympatric species of *Haminaea* (Opisthobranchia: Cephalaspidea). *J. Moll. Stud.* **57**:49-60.

Grahame, J. and G. M. Branch. 1985. Reproductive patterns of benthic marine invertebrates. *Oceanogr. Mar. Biol. Ann. Rev.* **23**:373-398.

Hadfield, M. G. and S. E. Miller. 1987. On developmental patterns of opisthobranchs. *Am. Malac. Bull.* **5**:197-214.

Hall, S. J. and C. D. Todd. 1986. Growth and reproduction in the aeolid nudibranch *Aeolidia papillosa* (L.). *J. Moll. Stud.* **52**:193-205.

Harrigan, J. F. and D. L. Alkon. 1984. Laboratory cultivation of *Haminoea solitaria* (Say, 1822) and *Elysia chlorotica* (Gould 1870). *Veliger* **21**(2):299-305.

Heller, J. and T. E. Thompson. 1983. Opisthobranch molluscs of the Sudanese Red Sea. *Linn. Soc. J. Zool.* **78**(4):317-348.

Hoagland, K. E. and R. Robertson. 1988. An assessment of poecilogony in marine invertebrates: Phenomenon or fantasy? *Biol. Bull. (Woods Hole, Mass.)* **174**:109-125.

Jablonski, D. and R. A. Lutz. 1983. Larval ecology of marine benthic invertebrates: Paleobiological implications. *Biol. Revs. Camb. Philos. Soc.* **58**:21-89.

Jackson, G. A. and R. R. Strathmann. 1981. Larval mortality from offshore mixing as a link between precompetent and competent periods of development. *Am. Nat.* **118**(1):16-26.

Kay, E. A. 1979. Mollusca. *In* Hawaiian Marine Shells: Reef and Shore Fauna Hawaii Sec. 4. Honolulu: Bishop Museun Press. pp. 424-428.

Leach, W. E. 1847. *Haminoea. In* On the classification of British Mollusca by Dr. W. E. Leach. Ed.: G. E. Gray. *Ann. Mag. nat. Hist.* **1**(20):267-273.

Lessios, H. A. 1987. Temporal and spatial variation in egg size of 13 Panamanian echinoids. *J. Exp. Mar. Biol. Ecol.* **114**:217-239.

Levin, L. A. 1984. Multiple patterns of development in *Streblospio benedicti* Webster (Spionidae) from three coasts of North America. *Biol. Bull. (Woods Hole, Mass)* **166**(3): 494-508.

Levin, L. A. and D. V. Huggett. 1990. Implications of alternative larval development modes for the seasonal dynamics and demography of an estuarine polychaete. *Ecology* **71**:2191-2208.

Levin, L. A., J. Zhu and E. A. Creed. 1992. The genetic basis of life-history characters in a polychaete exhibiting planktotrophy and lecithotrophy. *Evolution* **45**(2):380-397.

MacNae, W. 1962. Tectibranch molluscs from southern Africa. *Ann. Natal. Mus.* **15**(16):183-192.

MacPherson, J. H. and C. J. Gabriel. 1962. Marine molluscs of Victoria. *In* National Museum of Victoria Handbook #2. Melbourne: Melbourne Univ. Press. pp.242-244.

McEdward, L. R. and F. S. Chia. 1991. Size and energy content of eggs from echinoderms with pelagic lecithotrophic development. *J. Exp. Mar. Biol. Ecol.* **147**:95-102.

Powell, A. W. B. 1979. New Zealand Mollusca. Auckland: Collins. p.275.

Qian, P. Y. and F. S. Chia. 1991. Fecundity and egg size are mediated by food quality in the polychaete worm *Capitella* sp. *J. Exp. Mar. Biol. Ecol.* **148**:11-25.

Richards, A. 1921. The egg laying habits of *Haminoea virescens* (Sby.). *Proc. Okla. Acad. Sci.*:27-31.

Roughgarden, J. 1989. Life history evolution. *In*. M. W. Feldman, *ed.*, Mathematical Evolutionary Theory, pp. 270-300. Princeton, NJ: Princeton Univ. Press.

Roughgarden, J., Y. Iwasa and C. Baxter. 1985. Demographic theory for an open marine population with space-limited recruitment. *Ecology*. **66**:54-67.

Rudman, W. B. 1971. On the opisthobranch genus *Haminoea* Turton and Kingston. *Pac. Sci.* **25**:545-559.

Stearns, S. C. 1976. Life history tactics: a review of the ideas. *Q. Rev. Biol.* **51**:3-47.

_____. 1977. The evolution of life history traits. *Ann. Rev. Ecol. Syst.* **8**:145-171.

Strathmann, R. R. 1986. What controls larval development? Summary statement for the evolution session. *Bull. Mar. Sci.* **39**:616-622.

Thompson, T. E. and G. H. Brown. 1976. British opisthobranch molluscs. *In* Synopses of the British fauna #8. London: Academic Press. pp. 24-25.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.* **25**:1-45.

Todd, C. D. 1979. The population ecology of *Onchidoris bilamellata* (L.) (Gastropoda: Nudibranchia). *J. exp. mar. Biol. Ecol.* **41**:213-255.

Todd, C. D. and J. N. Havenhand. 1988. Physiological ecology of *Adalaria proxima* (Alder *et* Hancock) and *Onchidoris muricata* (Müller) (Gastropoda: Nudibranchia). III. Energy budgets. *J. Exp. Mar. Biol. Ecol.* **118**:191-205.

Usuki, I. 1966. The life cycle of *Haloa japonica* (Pilsbry). *Sci. Rep. Niigata Univ. Ser. D Biol.* **3**:87-105.

West , H. H., J. F. Harrigon and S. K. Pierce. 1984. Hybridization of two populations of a marine opisthobranch with different developmental patterns. *Veliger* **26**(3): 199-206.

Chapter 2

Poecilogony in the opisthobranch mollusc *Haminaea callidegenita*.

INTRODUCTION

Poecilogony is an unusual life history pattern involving more than one mode of development in one species (Giard 1905). Variable development provides these rare species with the evolutionary advantage of being able to exploit the benefits of more than one life history "strategy". The existence of poecilogony in benthic marine invertebrates is controversial partly because it is infrequently encountered but also because most of the original cases have been disproven, most often on the grounds that observations were actually made on sibling or cryptic species or reflect atypical development resulting from laboratory conditions (reviewed by Hoagland & Robertson 1988; in molluscs, Bouchet 1989). The few cases where poecilogony has not been disproven occur in the opisthobranch molluscs and polychaete annelids. However, since both groups are well-known as difficult taxonomic subjects, these cases have been discounted by many authors (Hoagland & Robertson 1988).

Poecilogony in benthic marine invertebrates is best demonstrated by confirming that the study population is composed of a single species and that poecilogony occurs throughout the population. Description of poecilogony within one species involves either observation of variable development in the offspring of a single individual, or cross-breeding experiments including genetic identification of the species. Currently, the most widely cited example of poecilogony is the polychaete *Streblospio benedicti* (Levin 1984; Levin & Creed 1986; Levin et al 1987). In this polychaete, different individuals produce either planktotrophic or lecithotrophic offspring. Despite the fact that these individuals cross-fertilize and produce viable offspring under laboratory conditions, some authors have interpreted this form of development as involving hybridization of recently diverged sibling species rather than true poecilogony. Hoagland & Robertson (1988) suggested that allozyme studies would be the definitive test in this case, but this approach has provided inconclusive results in other studies (e.g., the gastropod *Rissoa* in which allele frequencies contradict morphological and life history data in two species; Rehfeldt 1968, Colognola et al., 1986). Observation of poecilogony in the offspring of a single individual would circumvent this difficulty.

Proposed examples of poecilogony may reflect laboratory artifacts or developmental abnormalities unless they can be found throughout the reference population and occur

independently of culture conditions. For example, Lenderking (1954) described poecilogony in the periwinkle *Littorina angulifera* in which larvae were either brooded or released at different phases of the lunar cycle. Gallagher & Reid (1974) were unsuccessful in repeating these observations and Reid (1986) later concluded that the pre-mature release of larvae from broods as observed by Lenderking was a response to disturbance. Such an example of pre-mature release of larvae in species that otherwise would exhibit direct development seems likely for several proposed cases of poecilogony where most offspring hatch as juveniles and a few early hatchlings are released as lecithotrophic larvae which metamorphose within hours of hatching (Gohar & Eisawy 1967; Schmekel & Kress 1977). To exclude potential artifacts, observations of poecilogony should include experimental manipulation of culture conditions, as well as demonstrate that poecilogony is widespread in the reference population. Perhaps more importantly, observation of poecilogony throughout a population also illustrates its ecological relevance to the species.

In *Haminaea callidegenita* (Opisthobranchia: Cephalaspidea), poecilogony occurs in almost every egg mass; therefore, it occurs in the offspring of single individuals as well as throughout the population. In this species, both lecithotrophic (non-feeding) veligers and juveniles hatch from a single egg mass (Gibson & Chia 1989). The ecological result is that each parent produces offspring with a higher dispersal potential as well as offspring that recruit immediately into the parental population. All embryos simultaneously pass through the same developmental stages. Approximately half the siblings/ egg mass metamorphose within the egg mass and hatch as juveniles, and the other half hatch as veligers and metamorphose after a planktonic period ranging from 1-30 d. Both types of hatchling are released throughout the entire hatching period (ranging from 1 - 12 d among egg masses). Larvae that metamorphose within the egg mass are induced by a compound found in the jelly matrix covering the eggs (Gibson & Chia 1989). The degree of intracapsular metamorphosis varies widely (ranging from 4-100 %), but was observed in every egg mass. It is unlikely that this mode of development is direct development in which a few larvae escape prematurely under laboratory conditions because of the long planktonic period of hatched veligers.

The objectives of this chapter are to examine poecilogony in *Haminaea callidegenita* in terms of: 1) the extreme variance observed among egg masses, and 2) the factors that may influence rates of intracapsular metamorphosis. Factors that potentially influence poecilogony include reproductive traits of the parents, egg mass characteristics, culture conditions, and differences among clutches of eggs. Results indicate that much of the overall variance arises from the embryos themselves and is independent of reproductive traits and culture conditions.

MATERIALS AND METHODS

A. Animal collection and culture

Haminaea callidegenita adults and egg masses were collected from Padilla Bay (48°30'N, 122°29'W) and maintained at the University of Washington, Friday Harbor Laboratory, both in Washington State, U. S. A.. Padilla Bay is at the head of a large estuarine system and is dominated by extensive mud flats and seagrass beds of both *Zostera marina* and Z. *japonica*. The extensive mud flats cause temperatures within the bay to be extreme relative to those in nearby channels (Cassidy & McKeen 1986). *H. callidegenita* adults and egg masses were collected from Z. *japonica* beds in the upper intertidal zone. *H. callidegenita* are primarily found in association with *Zostera* blades, surface sediments around *Zostera* roots, and mats of *Ulva* sp. (sea lettuce), but may also be found crawling across submerged surface sediments.

Adults were collected from Padilla Bay at the beginning of the spawning season, as determined by the first appearance of egg masses. In the laboratory, adults were kept in 1 L Tripour beakers with two 10 cm^2 vents of 500 µm Nitex mesh that allowed continuous seawater flow. Food was supplied as *Ulva* sp. attached to rocks collected from the mid-intertidal zone at Friday Harbor Laboratory. This provided *Haminaea* with a substrate from which to graze diatoms, detritus and *Ulva* pieces. *Zostera* was not supplied as a grazing surface because of its poor laboratory survival. Food was replaced and cages cleaned at 2 week intervals. Cages were checked for newly spawned egg masses daily. As *Haminaea* were collected as adults, I assumed each had mated before collection, and probably with several individuals. In the laboratory, *Haminaea* were paired for 24 h to allow mating at 2 week intervals, each time with a different individual. Differences in dorsal pigmentation allowed identification of individuals before re-isolation. As *Haminaea* are simultaneous hermaphrodites, egg masses were collected from every individual.

Egg masses (EM) were cultured in 50 ml of 1 µm filtered seawater at 17 C. Warm temperatures were necessary because Padilla Bay is generally warmer (mean of 16.5 C from June to August; Cassidy & McKeen 1986) than nearby channels (summer mean of 11.3 C). EM maintained at ambient laboratory temperatures (9-12 C) had a high incidence of abnormal development. Culture jars were cleaned with hot fresh water and culture water replaced twice weekly throughout the experimental period. Several life history traits were examined, including reproductive characteristics of spawning females and the percent of hatchlings released as juveniles. Adults spawn repeatedly over a several week period and all egg masses were collected (between 4-6 egg masses for most females, ranging from 1 to 12 overall).

B. Experimental procedures

Reproductive traits

Reproductive traits of 165 females were measured in the laboratory (76 in 1990, 89 in 1991) producing a total of 597 egg masses (EM). Adults were collected from Padilla Bay and were maintained in the laboratory from April to October 1990 and from May to September in 1991.

The reproductive traits considered are: 1) interval between spawning events (d) of each "female"; 2) embryonic period (d), or the number of days from oviposition to first day of hatching/ EM; 3) hatching period (d), or the number of days from onset to completion of hatching/ EM; 4) number of offspring hatched/ EM; and 5) the percent juveniles hatching/ EM. Throughout the hatching period, hatchlings were removed daily and scored as veligers or juveniles under a Wild M-5 microscope. The percent juveniles hatching/ EM is the percent of hatchlings released as juveniles from each mass (i.e., larvae undergoing intracapsular metamorphosis) throughout the hatching period and was subsequently arcsin transformed (Sokal & Rohlf 1981) for statistical analysis.

Fecundity/ adult was also considered as the number of egg masses and as the total number of eggs produced by each adult.

As the major trait of interest was intracapsular metamorphosis, the effect of each trait on the percentage of juveniles hatching from each egg mass was predicted using a Model I Regression (Sokal & Rohlf 1981; LaBarbera 1989).

Egg mass size

Blotted wet weight of egg masses was determined to the nearest mg. Egg mass size was also approximated by counting the number of hatchlings/ egg mass.

Effects of culture in flowing seawater

Egg masses were cultured in standing and in flowing seawater in the laboratory (1989) and in the field (1991) to assess the effects of culture conditions in determining the percentage of juvenile hatchlings. Egg masses used were at the gastrula stage at the onset of the experiment. For laboratory assays, egg masses were collected in September 1989 and 2 groups (12 egg masses each) were cultured at ambient seawater conditions (11-12C). Group 1 (flowing seawater) was cultured in ice cube trays in which the bottom of each chamber was replaced with 100 µm Nitex mesh (1 egg mass/ chamber). The trays were suspended in a glass dish filled with 1 µm filtered seawater with egg masses submerged at all times. The top of the tray was above the water line to avoid mixing among chambers. Filtered seawater was supplied from a reservoir via a manifold suspended above the center

of the culture tray so that seawater continuously flowed down through the chambers and across the egg masses. Flow rate was approximately 20 ml/ min·chamber; milk was used in an initial test to ensure that all chambers/ tray received similar flows of seawater. At the same time, egg masses were cultured in 50 ml of 1 µm filtered seawater (Group 2) in standing cultures as a control.

In June 1991, egg masses were cultured in a similar experiment conducted in the field. Egg masses were placed in plastic snap-cap vials (approximately 50 ml volume) that were either 1) intact, for "standing seawater" conditions, or 2) supplied with two 100 µm Nitex mesh vents (each 2 cm^2 in area) to allow continual seawater flow through the vial. Ten egg masses were used for each group. Vials were attached to a rack and covered with an open mesh (5 cm^2 holes) to prevent clogging of the vials by floating macroalgae and diatom rafts. The rack was suspended in Argyle Lagoon, San Juan Island. Argyle Lagoon was chosen because it is a sheltered, shallow lagoon with summer temperatures similar to those found in Padilla Bay. Argyle provides a habitat similar to Padilla Bay in that it is dominated by seagrass (*Zostera marina*) and contains a large population of *Haminaea vesicula* and *Melanochlamys diomedea*, two cephalaspidean opisthobranchs found with *H. callidegenita* in Padilla Bay. Unlike Padilla Bay, Argyle Lagoon does not drain at low tide and therefore egg masses did not become desiccated.

In both of these experiments, cultures were cleaned daily by removing the egg masses and scrubbing each chamber with a fine brush. Hatchlings were removed daily throughout the hatching period and scored as veligers or juveniles under a Wild M-5 microscope. Data were compared between culture treatments with an unpaired two-tailed t-test for each experiment (field or laboratory). Data from 1991 were also compared with egg masses cultured using the standard laboratory protocol (standing cultures in an incubator; described above).

Role of egg mass jelly as a metamorphic inducer

Egg mass jelly (EMJ) is known to induce intracapsular metamorphosis (Gibson & Chia 1989). The relative effects of embryos and EMJ in determining intracapsular metamorphosis were compared in a random block design with 1 block/ cell. Embryos were separated from each egg mass and subsampled into 6 groups of 20 veligers. Each subsample was treated with EMJ separated from one of six additional egg masses in the following design:

	EMJ Source (1 to 6)					
Embryo Source (A to F)	A1	A2	A3	A4	A5	A6
	B1	B2	B3	B4	B5	B6
	C1	C2	C3	C4	C5	C6
	D1	D2	D3	D4	D5	D6
	E1	E2	E3	E4	E5	E6
	F1	F2	F3	F4	F5	F6

In this design, each subsample of embryos/ clutch was treated with EMJ from a different source (1 clutch = 1egg mass). Data were analyzed with a 2 way ANOVA using Systat. Two experiments were run (March and July 1990).

RESULTS

A. Description of poecilogony in *Haminaea callidegenita*

Haminaea callidegenita produce two kinds of offspring from every egg mass: approximately half hatch as lecithotrophic veligers and the rest metamorphose within the egg mass and hatch as juveniles (Gibson & Chia 1989). Development of all offspring appears to be identical throughout the 14 d encapsulated period until approximately 24 h before hatching occurs, at which point some offspring metamorphose within the egg mass. Both veligers and juveniles hatch simultaneously throughout the entire hatching period (4-5 d; Table 2.1). Embryonic and larval development has been described previously (Gibson & Chia 1989). In the present study, the mean percentage of juveniles hatching from each egg mass was approximately 61 and 69 % (1991 and 1992 respectively; Table 2.1), ranging from 4 - 100 % juveniles/ EM (Figure 2.1). The following experiments were designed to test for effects of factors that potentially contributed to the extreme variance among egg masses.

B. Factors influencing the percentage of juvenile hatchlings

Reproductive traits

Embryonic period and number hatched/ EM were not significantly different between egg masses spawned in each of the two years; spawn interval, hatching period and percentage of juvenile hatchlings were all higher in 1991 ($p < 0.01$; Table 2.1). In 1990,

females produced more eggs ($p < 0.05$; Table 2.1), although the total number of egg masses produced by each adult was not different between the two groups.

Several traits had significant effects on the percentage of juvenile hatchlings, although in some cases the effects were different in the two years considered (Table 2.2). For example, the percentage of juveniles increased with spawning interval in 1990 and decreased in 1991. Differences between the two years were slight (Figure 2.2). The regressions of other traits on percentage of juveniles were similar in terms of overall scatter, and therefore presentation of results was limited to Table 2.2. Egg masses with longer embryonic periods had a higher percentage of juveniles (1990 only) and egg masses with a longer hatching period had a lower percentage of juveniles (both years). Larger egg mass also showed a lower percentage of juveniles (1990). In 1991, females with higher fecundity also produced more juvenile hatchlings (Table 2.2); in 1990, these effects were not significant. Although many of these data were statistically significant, the correlation coefficients for each regression were low ($r^2 < 0.1$) suggesting that the effects considered here may be of relatively little biological importance in determining the large overall variance.

Egg mass size

Egg masses spawned in 1991 weighed approximately 0.65 mg and contained approximately 300 larvae/ EM (Table 2.3). Larger egg masses contained more larvae, and wet weight accounted for approximately a third of the observed variance in number hatched (Model I regression of effects of EM weight on clutch size: df=1, 84, F=36.48, p=0.0001, r^2=0.31). Larger clutches had a lower incidence of juvenile hatchlings (Table 2.3). Larger EM (wet weight) also released fewer juveniles but this relationship was not significant.

Effects of culture in flowing seawater

Culture in flowing seawater did not affect the percentage of juvenile hatchlings in laboratory experiments (Figure 2.3). Results were the same in field experiments (Argyle lagoon; Figure 2.3). These data indicate that the percentage of offspring hatching as juveniles was not influenced by the artificially still conditions of laboratory cultures. Data from the field experiment were also compared with data from egg masses cultured with the standard culture procedure to assess the impact of the laboratory culture environment. Again, no significant differences were found among the three groups (Figure 2.3).

Effects of source of inducer vs embryos in determining overall variance

Embryos of different clutches had significantly different responses to the metamorphic inducer (EMJ; Table 2.4A). EMJ from different sources did not differ in inductive potential . This indicates that the variance observed among egg masses in the percentage of juvenile hatchlings arises from the embryos and is independent of source of metamorphic inducer. This experiment was repeated with the same result (Table 2.4B).

DISCUSSION

Poecilogony in *Haminaea callidegenita* occurs as a dispersal polymorphism in which every parent produces both veliger and juvenile offspring. This highly unusual development mode depends on the time of metamorphosis (spanning a 30 d period including pre- and post-hatching periods; pers. obs.) and the presence of a metamorphic inducer (egg mass jelly) before hatching (Gibson & Chia 1989). Place of metamorphosis determines the percentage of offspring that hatch as juveniles and ranges from 4-100 % among the 600 egg masses observed.

Although embryological and reproductive traits did influence the percentage of juvenile hatchlings/ EM, this model accounted for a small proportion ($r^2 < 0.10$) of the overall variance observed (almost 100 %). Development time (embryonic and hatching periods) had slight and contrasting effects on the percentage of juveniles hatching. In one year only, egg masses with a longer embryonic period showed a higher incidence of juveniles but the correlation coefficient for this relationship was very small. In both years, egg masses with longer hatching periods had a lower incidence of intracapsular metamorphosis. This suggests that high proportions of juveniles in some egg masses does not occur simply as an effect of a longer encapsulated period, as might be expected as juveniles represent a later ontogenetic stage. It is possible that intracapsular metamorphosis and ability to hatch are related, perhaps through similar physiological pathways or through determination at the same ontogenetic stage. Therefore, egg masses with a short hatching period would also have a high incidence of intracapsular metamorphosis, as was observed although the correlation coefficients were low.

The percentage of juveniles at hatching was not affected by egg mass size. Strathmann & Chaffee (1984; also, Chaffee & Strathmann 1984) observed in the opisthobranch *Melanochlamys* that the physical size of larger egg masses imposes developmental constraints on embryos possibly by changing rates of oxygen/ waste diffusion through the gelatinous matrix of the egg mass. However, the present study found that this effect, if present, did not influence hatching type. This result is also

supported by an earlier observation that the thickness of jelly covering the eggs had no effect (Gibson & Chia 1989).

Female fecundity had a slight effect on the percentage of juvenile hatchlings. In 1991, females that produced more eggs or more egg masses also produced proportionately more juveniles. The positive relationship between fecundity and juvenile offspring in 1991 may have masked the negative effect of clutch size (as observed in 1990) resulting in the lack of effect observed in 1991. Also in 1991, females that had more rapid spawning events produced fewer juveniles, although the effect of this relationship was small. There are several interpretations of these observations. Females with a higher fecundity may have a longer period of spawning activity and the observed relationship could reflect long term changes throughout the reproductive season of individuals. Also, as female size is positively correlated with fecundity (Chapter 5), the observed result may reflect differences in female size. I was unable to assess changes throughout the entire reproductive season of an individual in this experiment because animals were collected as adults and may have spawned prior to collection. The effects of female size and changes throughout the reproductive period of an individual will be discussed in Chapter 5.

Culture condition had no effect on rates of intracapsular metamorphosis. This observation was supported by both laboratory and field studies, with two conclusions. The first conclusion is that both veliger and juvenile *Haminaea* are released in the field, albeit under artificial conditions. The second is that intracapsular metamorphosis is not affected by physical agitation of egg masses or by an increased oxygen concentration that may be associated with flowing seawater conditions. This later conclusion differs from observations of Caroll & Kempf (1990) in the nudibranch *Berghia verrucicornis* who found that both veligers and juveniles hatch from egg masses if aerated during culture, but only veligers were released from egg masses maintained in standing cultures. Intracapsular metamorphosis is also unaffected by culture temperature, as has been demonstrated previously (Gibson & Chia 1989).

Assuming much of the overall variance is independent of physical egg mass characteristics, female spawning activity and culture conditions, the major remaining source of variance observed among egg masses is the egg mass itself. Poecilogony arises from two factors: variation in the onset of metamorphic competence within a clutch (before or after hatching) and the presence of a metamorphic inducer in the egg mass jelly. Partitioning the effects of these factors revealed significant differences among embryos from different clutches, accounting for 50-90 % of the observed variance. The large differences observed among clutches of eggs suggests that the overall variance arises from the embryos. The genetic basis for this observation will be discussed in Chapter 5. There

were no significant differences among inducer sources (EMJ from different egg masses). This observation does not eliminate the possibility that different females produce different amounts of metamorphic inducer, but rather it suggests that if these differences occur they were not strong enough to be detected in this bioassay. This would not be unusual as metamorphic inducers are known to be active in trace amounts (Pawlik 1992) and it is likely that inducer concentrations inside the egg mass would exceed such amounts.

Conclusion

Every individual *Haminaea callidegenita* has poecilogonous development and produces both dispersive (non-feeding veliger) and non-dispersive (juvenile) offspring. The long planktonic period of hatched veligers (30 d) suggests that this hatching pattern is typical and that it is unlikely that poecilogony in this species reflects direct development with a few veligers being released prematurely. This hatching dichotomy occurred in almost every egg mass observed indicating that it occurs throughout the population. The percentage of juvenile hatchlings is highly variable among egg masses and is most strongly influenced by the embryos themselves rather than extrinsic sources, such as source of metamorphic inducer, egg mass characteristics, female reproductive characteristics and culture conditions.

Table 2.1: Summary of reproductive traits of *Haminaea callidegenita* maintained in the laboratory in 1990 and 1991 (different individuals in each year). Data listed are means and results of an unpaired two-tailed t-test comparing years. In A, n = number of egg masses; in B, number of females.
***p<0.001, **p<0.01, *p<0.05

						YEAR COMPARISON	
TRAIT	YEAR	n	X±SD	min	max	t-value	p
A. Individual egg masses							
Spawn interval (d)	1990	288	7.63 ± 0.29	0	36	-2.89	**
	1991	309	8.94 ± 0.35				
Embryonic period (d)	1990	288	14.62 ± 0.09	10	21	-0.74	ns
	1991	309	14.72 ± 0.10				
Hatching period (d)	1990	288	4.07 ± 0.11	1	12	-5.95	***
	1991	309	4.94 ± 0.10				
Number hatched/ EM	1990	288	341.28 ± 8.83	27	907	1.19	ns
	1991	309	326.65 ± 8.75				
% Juveniles/ EM	1990	288	61.11 ± 1.43	4.04	100	-4.75	***
	1991	309	69.62 ± 0.54				
B. Fecundity/ female							
#Egg masses	1990	42	4.21 ± 0.45	1	12	1.48	ns
	1991	47	3.38 ± 0.87				
Total # eggs	1990	42	1646.97± 215.55	63	5686	2.42	*
	1991	47	1074.36 ± 293.16				

Table 2.2: Effect of reproductive traits on percentage of juvenile hatchlings in *Haminaea callidegenita* . Data presented are the results of a Model I regression of the percentage of juveniles on other life history traits for each egg mass; data from 1991 and 1992 are considered separately. In A, df refer to number of egg masses; in B, to number of females. ***p<0.001, **p<0.01, *p<0.05

TRAIT	YEAR	df	F	p	r^2	
A. Individual egg masses						
Spawn interval (d)	1990	1, 288	4.42	*	0.01	y= 0.34x+49.95
	1991	1, 309	6.71	**	0.02	y=–0.32x+60.62
Embryonic period (d)	1990	1, 288	12.05	***	0.04	y= 2.05x+22.54
	1991	1, 309	2.90	ns	0.01	
Hatching period (d)	1990	1, 288	17.46	***	0.06	y=–2.24x+61.68
	1991	1, 309	7.66	**	0.03	y=–1.09x+63.16
Number hatched/ EM	1990	1, 288	17.27	***	0.05	y=–0.03x+61.33
	1991	1, 309	2.12	ns	0.01	
B. Fecundity/ female						
#Egg masses	1990	1, 41	1.74	ns	0.06	
	1991	1, 46	5.14	*	0.10	y = 1.03x+48.36
Total # eggs	1990	1, 41	2.08	ns	0.06	
	1991	1, 46	4.56	*	0.09	y =0.003x+48.73

Table 2.3: Egg mass size and its effects on the percentage of juveniles hatching/ mass. A. Means (±S.E.) for egg masses produced by laboratory maintained *Haminaea callidegenita* (1991). B. Results of a Model I regression of effects of egg mass size on the percentage of juveniles (as arcsin transformed percentages). ***p<0.001

A. Egg mass size

TRAIT	n	X±S.E.	min	max
# Hatched/ EM	162	310.62±9.31	69.00	700.00
Wet weight (mg)	88	0.65±0.02	0.31	1.65

B. Effects of egg mass size on percentage of juvenile hatchlings

TRAIT	df	F	p	r^2	
# Hatched/ EM	1, 161	17.01	***	0.10	y= –0.03x+81.76
Wet weight (mg)	1, 84	0.06	ns	0.00	y= –1.56x+71.56

Table 2.4: Effects of source of embryos and of metamorphic inducer in determining the percentage of juvenile hatchlings in *Haminaea callidegenita*. Subsamples of embryos from each egg mass were treated with EMJ from different sources, for a total of 6 egg masses in March (A) and 5 egg masses in July 1990 (B). Data were analysed with a two-way ANOVA using arcsin transformed percentages. ***p<0.001

A. Experiment I: March 1990

Percent juveniles X±S.E. 54.81±3.83%

n=36 r=0.70 r^2=0.49

Source	df	SS	MS	F	p
Embryos	5	1.529	0.306	4.174	***
Metamorphic Inducer	5	0.235	0.047	0.642	ns
Error	25	1.832	0.073		

B. Experiment II: July 1990

Percent juveniles X±S.E. 65.67±6.12%

n=20 r=0.93 r^2=0.87

Source	df	SS	MS	F	p
Embryos	3	2.255	0.752	23.739	***
Metamorphic Inducer	4	0.290	0.072	2.288	ns
Error	12	0.380	0.032		

Figure 2.1. Variability in the proportion of juveniles hatching from *Haminaea callidegenita* egg masses. Data are the percent juveniles/ total hatchlings plotted against clutch size (n = 377 egg masses).

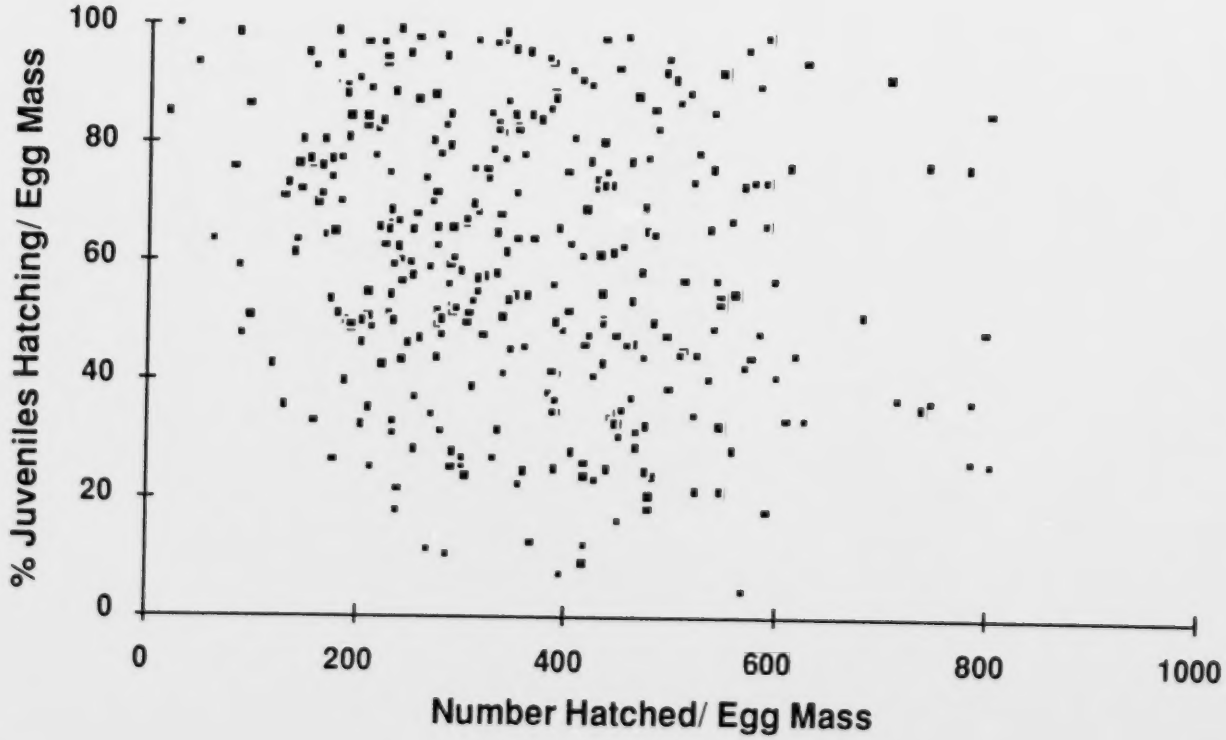


Figure 2.2. The effect of spawning interval on the percent juveniles (arcsin transformed) hatching from each egg mass, plotted with the results of a linear regression. A. Data from 1990, B. from 1991.

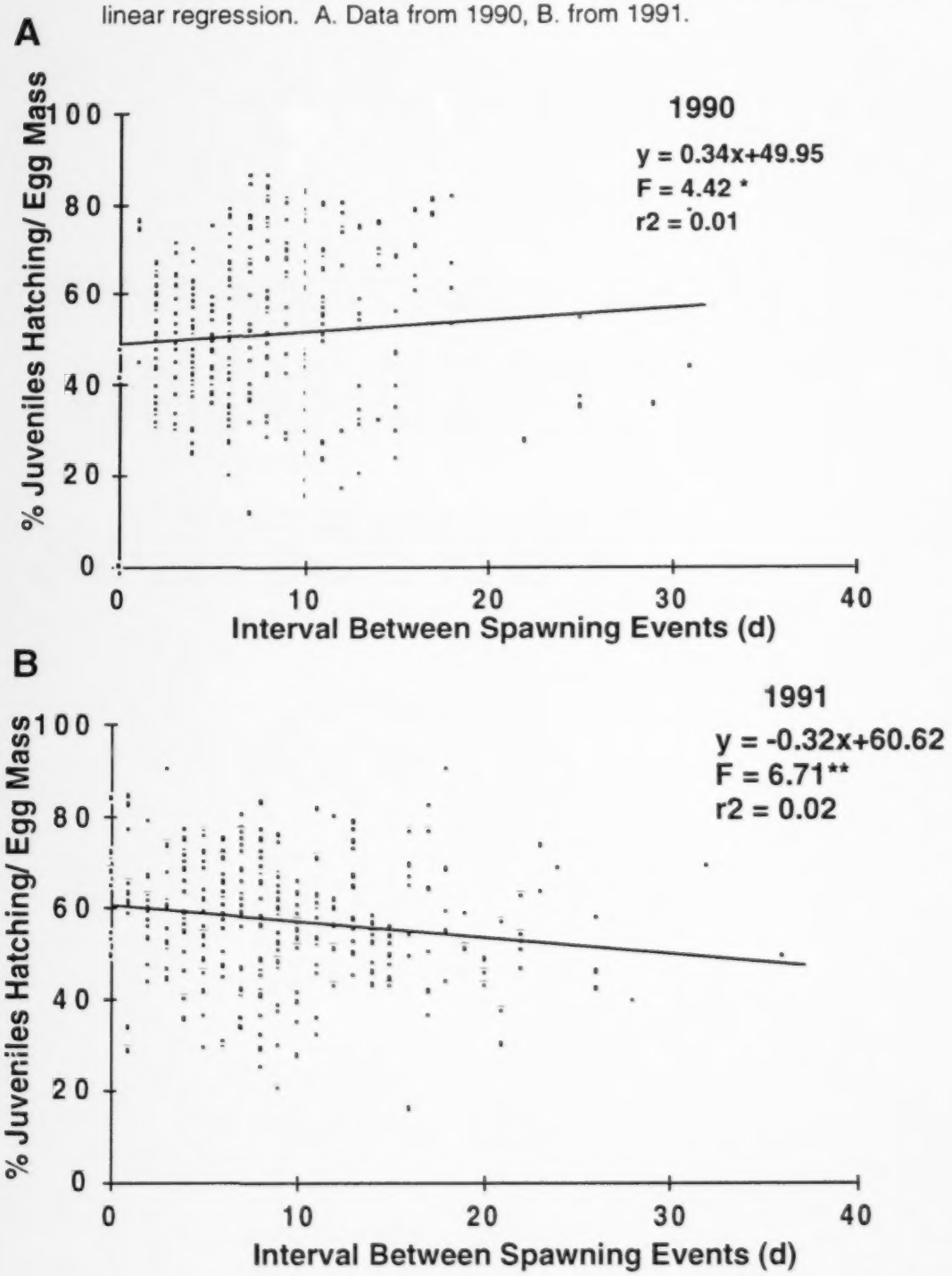
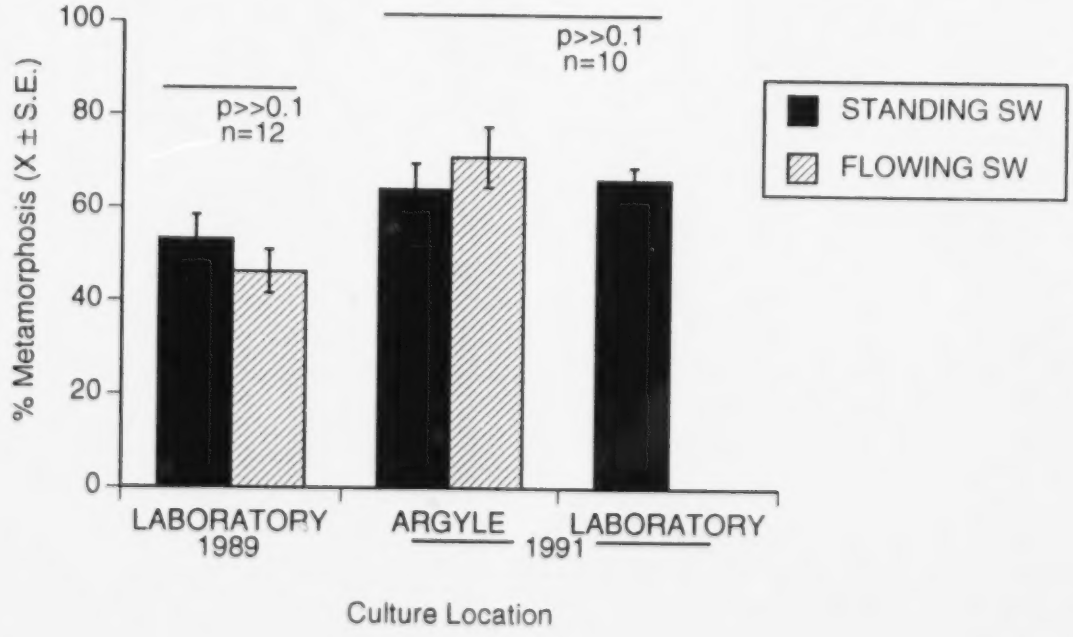


Figure 2.3. The effect of culture conditions on the percent juveniles hatching from egg masses maintained in standing (solid bars) or flowing (hatched bars) seawater conditions. 1989: egg masses were maintained in the laboratory. 1991: egg masses maintained in Argyle Lagoon (middle columns) as well as laboratory observations (far right). Bars above columns indicate no significant differences were found in each experiment (1989, unpaired two-tailed t-test;1991, one way ANOVA).



Literature Cited

Bouchet, P. 1989. A review of poecilogony in gastropods. *J. Moll. Stud.* **55**:67-78.

Carroll, D. J. and S. C. Kempf. 1990. Laboratory culture of the aeolid nudibranch *Berghia verrucicornis* (Mollusca, Opisthobranchia): Some aspects of its development and life history. *Biol. Bull.* **179**: 243-253.

Cassidy, P. M. and G. L. McKeen. 1986. Padilla Bay baseline water quality record. Report to NOAA/ OCRM/ MEMD by Western Washington University, Shannon Point Marine Center. 472pp. Anacortes, Washington. Padilla Bay National Estuarine Research Reserve Reprint Series No. 2, 1990.

Chaffee, C. and R. R. Strathmann. 1984. Constraints on egg masses. I. Retarded development within thick egg masses. *Biol. Bull.* **84**:73-83.

Colognola, R., P. Masturzo, G.F. Russo, M. Scardi, D. Vinci and E. Fresi. 1986. Biometric and genetic analysis of the marine rissoid *Rissoa auriscalpium* and its ecological implications. *Marine Ecology* **7**:133-140.

Gallagher, S. and G. Reid. 1974. Reproductive behavior and early development in *Littorina scabra angulifera* and *Littorina irrorata* in the Tampa Bay region of Florida. *Malacological Review* 7: 105-125.

Giard, A. 1905. La Poecilogonie. *Comp. Rend. Six. Congr. Internationale de Zool.* Berne, 1904: 617-646.

Gibson, G. D. and F. S. Chia. 1989. Developmental variability (pelagic and benthic) in *Haminaea callidegenita* (Opisthobranchia: Cephalaspidea) is influenced by egg mass jelly. *Biol. Bull. (Woods Hole, Mass.)* **176**:103-110.

Gohar, H. A. F. and A. M. Eisawy. 1967. The egg masses and development of five rachiglossan prosobranchs (from the Red Sea). *Publ. Marine Biol. Station Al-Ghardaqa Egypt.* **14**:216-266.

Hoagland, E. and R. Robertson. 1988. An assessment of poecilogony in marine invertebrates: phenomenon or fantasy? *Biol. Bull.* **174**: 109-125.

La Barbera, M. 1989. Analyzing body size as a factor in ecology and evolution. *Annu. Rev. Ecol. Syst.* **20**:97-117.

Lenderking, R. E. 1954. Some recent observations on the biology of *Littorina angulifera* Lam. of Biscayne and Virginia Keys, Florida. *Bull. Mar. Sci. Gulf Caribb.* **3**:273-296.

Levin, L. A. 1984. Multiple patterns of development in *Streblospio benedicti* Webster (Spionidae) from three coasts of North America. *Biol. Bull.* **166**(3):494-508.

Levin, L. A., H. Caswell, K. D. DePatra, and E. L. Creed. 1987. Demographic consequences of larval development mode: Planktotrophy vs. lecithotrophy in *Streblospio benedicti*. *Ecology* **68**:1877-1886.

Levin, L. A. and E. L. Creed. 1986. Effects of temperature and food availablility on reproductive responses of *Streblospio benedicti* with planktotrophic or lecithotrophic development. *Mar. Biol.* **92**:103-113.

Pawlik, J. R. 1992. Chemical ecology of the settlement of benthic marine invertebrates. *Oceanogr. Mar. Biol. Annu. Rev.* **30**:273-335.

Rehfeldt , N. 1968. Reproductive and morphological variations in the prosobranch "*Rissoa membranacea*." *Ophelia* **5**(1):157-173.

Reid, D. G. 1986. *The Littorinid Molluscs of Mangrove Forests in the Indo-Pacific Region. The Genus* Littoraria. British Mus. (Nat. Hist.), London. 228 pp.

Schmekel, L. and A. Kress. 1977. Die Gattung *Doto* (Gastropoda: Nudibranchia) im Mittelmeer und Armelkanal, mit Beschreibung von *Doto acuta*, n. sp. *Malacologia* **16**(2):467-499.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry: the Principles and Practice of Statistics in Biological Research. 2nd ed. New York: W. H. Freeman and Co. 859pp.

Strathmann, R. R. and C. Chaffee. 1984. Constraints on egg masses. II. Effect of spacing, size and number of eggs on ventilation of embryos in jelly, adherent groups, or thin-walled capsules. *Biol. Bull.* **84**: 85-93.

Chapter 3

Metamorphosis in the opisthobranch *Haminaea callidegenita*: competence, delay and the effects of metamorphic inducers.

INTRODUCTION

The planktonic larva is the primary dispersal stage in most species of benthic marine invertebrates (Thorson 1950). In consequence, the length of the larval period affects the colonization of new populations (Day & McEdward 1984), gene flow among existing populations (Scheltema 1986), and extinction and speciation events (Jablonski & Lutz 1983). The length of the larval period ranges from hours to months among species (Thorson 1950), and is known to vary within species as well, when modified either by larval environment (Pechenik 1984; Paulay *et al.* 1985; Zimmerman & Pechenik 1991) or by the ability of competent larvae to delay metamorphosis (reviewed by Pechenik 1990). Metamorphosis ends the larval period and is determined by two events: larvae must first become physiologically competent to metamorphose and second, must intercept a cue identifying a suitable habitat, known as a metamorphic inducer.

Both of these events have been much studied (see reviews by Pechenik 1990 on delay of metamorphosis, and Pawlik 1992 on metamorphic inducers) but only certain aspects have been addressed. The rate at which metamorphosis occurs throughout the entire larval period (how many larvae/ clutch become competent on any day) and variability in time of competence among offspring of different parents is not often addressed. Time of competence has frequently been studied as onset of competence of the first larvae/ culture (Bayne 1965; Hadfield 1978; Harrigan & Alkon 1978; Pechenik 1984; Lima & Pechenik 1985), or as the ability of larvae to delay metamorphosis in the absence of a metamorphic inducer (Bayne 1965; Pechenik 1990; Pechenik et al. 1993). Larvae that fail to metamorphose in the same time interval as their faster-developing counterparts are generally discarded from the experiment. Rather than reflecting "inferior" larvae or less efficient feeders, this variability in time of competence may have major implications for larvae of one clutch by making the duration of the dispersal stage more flexible, as has been suggested for larvae that maintain competence (Birkeland *et al.*, 1971; Pechenik 1980; Jackson & Strathmann 1981; Chia & Koss 1988) or delay metamorphosis over long periods (reviewed by Pechenik 1990).

The second factor necessary for metamorphosis to occur in most species is the presence of a metamorphic inducer. Inducers are species-specific chemical or physical cues

that are characteristic of a habitat suitable for juvenile or adult life. Inducing substrates have been identified for many species and often have been isolated to a single substrate (reviewed by Pawlik 1992). Competent larvae that are denied an appropriate inducer may delay metamorphosis until an appropriate cue is intercepted (up to 300 d in the planktotrophic opisthobranch *Aplysia*; Kempf 1981; 1 year in the lecithotrophic seastar Mediaster, Birkeland *et al.* 1971) or, more commonly, will undergo "spontaneous" metamorphosis in absence of an observed cue (Highsmith & Emlet 1986), or exhibit a decrease in "choosiness" and settle on a perhaps less favorable substrate (Thorson 1950; Knight-Jones 1953; Crisp 1974, 1988; Rumrill 1989). Again, a decrease in larval "choosiness" may provide larvae with a great deal of flexibility in encountering a suitable substrate, as long as the preferred habitat is not highly specific. A decrease in "choosiness" may indicate changing metamorphic requirements throughout the metamorphic period; however, empirical evidence to support this hypothesis is lacking.

In this study, I examined the onset of metamorphic competence and the importance of several natural inducers throughout the metamorphic period in the sea slug *Haminaea callidegenita* (Opisthobranchia: Cephalaspidea). *H. callidegenita* has variable development in which metamorphosis can occur within the egg mass or after hatching. Time of metamorphosis therefore determines the proportion of offspring that disperse and those that recruit directly to the parental population. Metamorphosis is induced by a compound found in the gelatinous matrix covering the developing larvae (Gibson & Chia 1989). In this species, larvae metamorphosed gradually over a period of 1-14 d in continuous presence of an inducer. Time of metamorphosis was exclusive of food effects, as these are non-feeding larvae. The specific objectives were to describe 1) variable onset of metamorphic competence throughout the metamorphic period (pre- and post-hatching), 2) the ability of *H. callidegenita* larvae to delay metamorphosis and 3) the increasing sensitivity of *H. callidegenita* larvae to various metamorphic inducers throughout the metamorphic period.

MATERIALS AND METHODS

A. Animal collection and culture

Haminaea callidegenita adults and egg masses were collected in the summer of 1992 from Padilla Bay in Washington State. Culture procedures followed those described previously for adults and egg masses (Chapter 2).

B. Time of metamorphosis

Time of competence throughout the larval period

In this study, competence is defined as the physiological ability of larvae to metamorphose. This ability was recorded as metamorphosis in response to the natural inducer found in egg mass jelly (EMJ) and to excess potassium, known to have inductive potential on competent larvae of many marine invertebrate taxa (Pechenik & Heyman 1987).

Pre-hatching metamorphosis

The ability of several natural substrates to induce metamorphosis before hatching was assessed by treating encapsulated larvae with each substrate. Gastrulae were separated from egg masses by teasing apart the mass with fine forceps. Embryos were rinsed in 1 µm filtered seawater (fSW) several times to remove all traces of egg mass jelly (EMJ). Embryos were placed in 2 ml Falcon wells containing fSW and a single metamorphic inducer as described below. Cultures were maintained in an incubator at 17 C in constant light (necessary as light was the heat source for the incubator). Warm culture conditions correspond to the ambient temperature in Padilla Bay which is warmer (mean of 16.5 C from June to August) than nearby open water (summer mean of 11.3 C; Cassidy & McKeen 1986). Cultures were cleaned twice weekly by transferring embryos to new culture wells containing fSW and fresh metamorphic inducer. At 24 h intervals throughout the hatching period, hatched veligers and juveniles were counted under a Wild M-5 microscope and removed from the culture. Hatchlings were scored as veligers if velar lobes were intact and ciliated. Juveniles had lost cilia and velar lobes were resorbed. Unlike nudibranchs, the shell is retained through the adult stage. The percent intracapsular metamorphosis was determined for the entire hatching period and subsequently arcsin transformed for statistical analysis (Sokal & Rohlf 1981).

Opisthobranch veligers are often induced to metamorphose by a prey species (Clark 1975; Hadfield & Miller 1987). I tested several substrates for their ability to induce intracapsular metamorphosis in *Haminaea callidegenita*; substrates selected were often associated with juveniles in the field (pers. obs.). Substrates included: 1) egg mass jelly (= EMJ), a known metamorphic inducer in this species (approximately 1 mm^3/ 2 ml well; Gibson & Chia 1989); 2) surface sediment (1 drop dilute suspension/ well); 3)*Chaetamorpha linum*, a filamentous green alga (2 x 5mm filaments/ well); and 4) the seagrass *Zostera marina* (0.5 cm^2 section of blade/ well). Potentially active substrates were freshly collected from Padilla Bay, except for Chaetamorpha which was collected from Spencer Spit. Excess potassium in seawater (19 mmol K^+) was also tested as this is

known to induce metamorphosis in many benthic marine invertebrates (Yool *et al.* 1986). Twenty veligers were cultured/ well for 15 replicates/ substrate (8 for *Chaetamorpha*).

Post-hatching metamorphosis

Metamorphosis after hatching was induced by several substrates. Veligers were collected and pooled within 24 h of hatching from intact egg masses. Only veligers hatched from intact egg masses were used (*vs.* those separated from EMJ earlier in development) to limit the analysis to veligers that would normally metamorphose after hatching. Ten veligers were subsampled and placed in 2 ml Falcon wells containing one of the following substrates: 1) 1 µm filtered seawater (SW; control for absence of metamorphic inducer; 10 cultures); 2) SW + EMJ (n = 10); 3) SW + *Chaetamorpha linum* (n = 6); 4) SW + *Zostera marina* (n = 6); 5) SW + sediment (n = 6); and 6) SW with excess potassium (19mmol K^+; n = 10). Potential inducers were added as described for intracapsular metamorphosis. The number of metamorphosed juveniles was counted at 24 h intervals throughout the post-hatching larval period (15 d).

Variable induction of intracapsular metamorphosis among clutches

Induction of metamorphosis was also compared using embryos from different clutches. Embryos were separated from 13 egg masses, each at the gastrula stage. Embryos were rinsed in filtered seawater to remove all traces of EMJ then subsampled into 6 groups of 30 eggs each. Two subgroups of embryos from each clutch were cultured in seawater only (metamorphic inducer absent), two in the presence of EMJ and two in 19 mMol K^+. The sensitivity of larvae from each clutch to both sources of metamorphic inducer (EMJ, 19 mMol K^+) was examined with a one-way ANOVA. The relative influence of egg mass *vs* inducer in determining the percent intracapsular metamorphosis was determined with a two way ANOVA. Percentage data were arcsin transformed prior to analysis (Sokal & Rohlf 1981).

RESULTS

Time of metamorphosis throughout the larval period

Pre-hatching metamorphosis

Veligers cultured in the presence of EMJ showed the highest percent metamorphosis (53 %) at the end of the 3 d hatching period (Figure 3.1). Veligers cultured in the absence of metamorphic inducer (seawater only) showed only 7 % metamorphosis.

Rates of metamorphosis increased throughout the hatching period in response to all substrates tested (Figure 3.1).

Substrates differed in their potential to induce metamorphosis before hatching (Table 3.1A). The percent metamorphosis of veligers cultured with sediment or *Chaetamorpha linum* were very slightly higher throughout the hatching period than embryos cultured in the absence of inducer (to 10 % metamorphosis; Figure 3.1); these differences were not significant (Table 3.1B) suggesting that these substrates did not induce metamorphosis above that spontaneously occurring in seawater. EMJ and seawater with elevated K^+ levels induced metamorphosis at a significantly higher rate throughout the hatching period than occurred in seawater. There were no significant differences in the percent metamorphosis induced by EMJ and K^+ (Table 3.1B). *Zostera marina* induced intracapsular metamorphosis to an extent intermediate between absence of inducer and presence of EMJ, although this was evident at the end of the hatching period (38 % on d 3; Figure 3.1). Induction by *Zostera* were significantly different from both SW and EMJ (Table 3.1B) indicating that although *Zostera* will induce intracapsular metamorphosis, fewer larvae will respond and the effect will be delayed relative to rates of metamorphosis observed in response to EMJ.

Post-hatching metamorphosis

Substrates differed in the potential to induce metamorphosis in hatched veligers. The most rapid onset of metamorphosis after hatching occurred in veligers cultured in the presence EMJ or in 19 mMol K^+ (e.g., on d 1; Table 3.2). Half of these veligers had completed metamorphosis by the second day of the experiment and metamorphosis was completed by day 11. Although *Chaetamorpha linum* failed to induce intracapsular metamorphosis, it did induce metamorphosis after hatching (Table 3.2). Rates of metamorphosis induced by *Chaetamorpha* were similar to that induced by EMJ in that metamorphosis was completed at the same time although the initial metamorphosis was delayed (50 % of the veligers tested metamorphosed by day 3-4). Veligers cultured with *Zostera marina* were also slower to begin metamorphosis but completed metamorphosis at the same time as veligers in the presence of EMJ. Metamorphosis of veligers cultured in the absence of inducer (SW only) showed a steady low rate of metamorphosis throughout the planktonic period and could delay metamorphosis 4-5 d longer than veligers cultured with inducer. Sediment did not induce metamorphosis more than that observed in SW. Variances associated with mean time of metamorphosis (Table 3.2) are similar to those observed in intracapsular metamorphosis of intact egg masses (e.g., mean $\pm$ standard error of 61 $\pm$ 7.27 % juveniles, n = 10 egg masses).

Time of competence throughout the metamorphic period (pre- and post-hatching)

Cumulative rates of metamorphosis were compared throughout the metamorphic period (i.e., from first to last metamorphosis per clutch). Rates of metamorphosis after hatching were adjusted to reflect the percent veligers that had metamorphosed before hatching in response to the same substrate (Figure 3.2). Adjusting the data in this way may introduce some error as data collected before and after hatching did not come from the same egg masses which may have influenced the overall variance in time of metamorphic competence (Chapter 2 and below). However, all assays were done at the same time on larvae from egg masses collected from the same population.

Veligers treated with EMJ and K^+ showed the same rates of metamorphosis throughout the metamorphic period (Figure 3.2). Veligers cultured in SW or in the presence of *Chaetamorpha* showed very low levels of intracapsular metamorphosis but their effects diverged after hatching. Veligers in SW maintained the lowest rate of metamorphosis while *Chaetamorpha* induced much higher rates of metamorphosis post-hatching. *Zostera* induced metamorphosis at rates intermediate to both the presence and absence of inducer (EMJ/ SW; Table 3.1) before hatching and remained so throughout most of the metamorphic period (Figure 3.2). Rates of metamorphosis in response to sediment were similar to those in seawater.

Metamorphosis was also compared as percent metamorphosis per day for veligers cultured in the presence of SW and EMJ (Figure 3.3). The daily rate of metamorphosis was initially high in the presence of EMJ (35 %) and low throughout the rest of the hatching period. A second, smaller peak in metamorphosis occurred in veligers after hatching (d 4), followed by a gradual decrease throughout the larval period. Although competence occurred over a long period (14 d), all larvae completed metamorphosis. Veligers cultured in SW (inducer absent) showed lower daily rates of metamorphosis throughout the experimental period with a peak response at d 6 (Figure 3.3). This may represent "spontaneous" metamorphosis that has been noted in other species (Pechenik et al. 1993).

Variable induction of intracapsular metamorphosis among clutches

Intracapsular metamorphosis occurred at approximately the same rate in this assay (Table 3.3A) as observed above in response to EMJ, 19mmol K^+, and in seawater (Figure 3.1). The percent metamorphosis in seawater did not differ among clutches (=egg masses), although the overall incidence of metamorphosis was very low (5 %). Clutches did differ in response to EMJ or to K^+ as metamorphic inducers (one way ANOVA,

$p < 0.01$; Table 3.3B) with a range in mean response/ clutch from 31 - 91 % metamorphosis. Individual clutches also responded differently to induction by EMJ or K^+ (two factor ANOVA, $p < 0.01$; Table 3.3B) although the difference in mean response over all egg masses considered was very small (63% in response to EMJ, 58% to K^+) .

DISCUSSION

Length of the larval period and time of metamorphic competence

Length of the planktonic larval period has far-reaching ecological and evolutionary consequences (Jablonski & Lutz 1985, Scheltema 1986). There are many indirect reports of variability in the time the planktonic period is terminated by metamorphosis (success in achieving simultaneous metamorphosis in culture is seldom observed) but this variability is generally ascribed to variable physiological condition of eggs produced per spawning event or to larval performance in feeding and growth (Bayne 1965), if addressed at all. Most authors report primarily on the onset of competence in a culture. However, variable time of competence could provide additional flexibility in length of larval life that may allow some larvae/ clutch to locate a patchy, specific, or widely separated habitats (Jackson & Strathmann 1981; Pechenik 1990).

Haminaea callidegenita larvae from one clutch become competent to metamorphose gradually over a period of 14 d (including 3 d before hatching) in the continuous presence of a metamorphic inducer (EMJ). Throughout this period, daily rates of metamorphosis in *Haminaea callidegenita* changed from an initial high rate of metamorphosis within the egg mass (50 % veligers had metamorphosed by 3 d), followed by a second peak immediately after hatching, and continuing at a low rate of metamorphosis throughout the rest of the larval period (Figure 3.3). This pattern of metamorphosis also occurred in response to elevated K^+. Variable onset of competence within a clutch may provide an ecological advantage of providing a broader dispersal range for propagules produced by a population such that the majority metamorphose within or near the parental habitat and the number of "dispersers" rapidly decreases with time. All larvae/ clutch become competent and late developers do not represent physiologically "inferior" offspring, as may be inferred (perhaps wrongly) in other species in which observations of metamorphosis are not continued for all larvae in the experiment.

Whether this range in variability is common is unknown. It is difficult to compare these data with other species because observations by others have been made by "pulsing" subsets of larvae with inducer at various time intervals and few authors have followed larvae throughout the entire metamorphic period. Molluscs often show a metamorphic

profile with an initial slow rate of metamorphosis followed by a plateau at high rates over several days although metamorphosis is rarely completed by all larvae per culture (e.g., Hadfield & Scheuer 1985; Coon *et al.* 1990; Pechenik & Gee 1993). In *Haminaea callidegenita*, daily rates of metamorphosis appear to be quite different. Low rates of metamorphosis of the first larvae achieving competence, as observed in other molluscs, either did not occur in *H. callidegenita* or alternatively were not detectable because initial metamorphosis is rapid (within 24 h before hatching occurs; Gibson & Chia 1989) and occurs within the egg mass where it is difficult to observe (in cases where the velum is not extended). The initial peak in metamorphosis may correspond to an abbreviated plateau of high rates of metamorphosis, as observed in other molluscs. The lower daily rates of metamorphosis in the remaining metamorphic period might have been observed by other authors had observations been continued to end of the larval period.

In this experiment, I have assumed that larvae will metamorphose when they become physiologically competent to do so if a metamorphic inducer is present; therefore, time of metamorphosis indicates the time a larva becomes competent. Larvae of other molluscs are thought to become habituated to an inducer if continuously present (e.g., *Phestilla sibogae*, Hadfield & Scheuer 1985, Hirata & Hadfield 1986; *Haliotis rufesens*, Trapido-Rosenthal & Morse 1986 a&b; but not *Crepidula fornicata*, Pechenik and Gee 1993). Habituation is unlikely to play a major role in *Haminaea callidegenita* because 1) a certain proportion of veligers/ clutch are sensitive to the inducer (EMJ) throughout the encapsulated period despite the continued presence of jelly and, 2) it is not necessary to desensitize hatched larvae before they will respond, as occurs in *Phestilla*.

Variability in time of competence is also influenced by significant differences in the time intracapsular metamorphosis is observed among clutches (induced by EMJ). This suggests that variance in time of competence may be genetic, as is supported by the observation that the percent intracapsular metamorphosis is determined by the embryos themselves and is independent of inducer (Chapter 2). Variable time of competence within a culture (although not necessarily a clutch) has been observed in other species, but has generally been ascribed to environment or food availability (Hadfield 1977; Kempf & Hadfield 1985; Pechenik et al 1990). The genetic basis for time of metamorphosis in *Haminaea callidegenita* will be further addressed in Chapter 5.

Larvae from different clutches responded differently to the natural inducer (EMJ) and induction by elevated K^+, although the mean responses were very similar. This supports the hypothesis that K^+ is triggering metamorphosis in marine invertebrates via a different pathway than does the natural inducer (Yool *et al*, 1986; Pechenik & Gee 1993), possibly by depolarizing an excitable membrane in the nervous system (Pechenik &

Heyman 1987) rather than contacting an external receptor organ and eliciting a cascade effect, as has been suggested for the natural inducer (Burke 1983; Arkett et al 1989).

Metamorphosis inducing activity of different substrates

Haminaea callidegenita larvae metamorphosed in response to several substrata throughout the metamorphic period. The effects of these natural inducers changed throughout the metamorphic period. This could represent either a decrease in larval "choosiness" as has been proposed for competent larvae of other species if denied a "preferred" substrate (Thorson 1950, Knight-Jones 1953; Crisp 1988; Rumrill 1989; Pechenik 1990) or alternatively, an increased sensitivity to other cues which may identify an acceptable habitat. Metamorphosis was not induced by sediment indicating that the decrease in "choosiness" is not completely haphazard in this species and although larvae will gradually respond to an increasing assortment of substrates, they still express some selectivity.

For example, larvae metamorphosed in response to EMJ as soon as they became competent throughout the metamorphic period (time of competence supported by larval response to elevated K^+). The initial sensitivity to EMJ was high and as jelly would always be present during the encapsulated phase in nature, this would result in a high incidence of juvenile hatchlings. Post-hatching sensitivity gradually decreased throughout the metamorphic period. The probability of a hatched veliger encountering an egg mass in nature is low even in Padilla Bay with its extensive population of *Haminaea* as well as dense seagrass beds that may create hydrologic conditions that favor retention of larvae within the bay. Also, such an encounter would not necessarily provide a larva with a reliable cue of a recruitment site containing an established *H. callidegenita* population because EMJ of other species will have the same effect regardless of habitat of the test species (Chapter 4). In nature, it is likely that other cues (chemical or physical) also play a role in identification of a suitable site (e.g., as is known in barnacles; Crisp 1974). A possibility is water temperature, as *H. callidegenita* adults are found in bays with slightly warmer temperatures than other bays in the vicinity (pers. obs.).

Veligers showed no sensitivity to *Chaetamorpha linum* before hatching yet rapidly responded to this alga post-hatching after an initial (1-3 d) delay before peak response. Metamorphosis in response to *Zostera* was intermediate to that induced by EMJ and SW throughout the metamorphic period. An ecological advantage for veligers to respond to *Zostera* before hatching and not to *Chaetamorpha* is not clear. In the field, both substrates are associated with egg masses as well as with small juveniles indicating that they both provide sites adequate for adult and juvenile existence. However, *Zostera* beds are much

more extensive in this habitat. Whether veligers respond to *Zostera* before hatching in nature is not known but its effect would likely be small relative to that of EMJ which is in closer proximity to developing embryos. The response of hatched veligers to *Zostera* was similar to their response to *Chaetamorpha* - there was an initial period of low metamorphosis (1-3 d) followed by rapid metamorphosis. This post-hatching delay period may allow sufficient time for short range dispersal, i.e., to another part of the bay or of the estuarine system.

Delay of metamorphosis

Haminaea callidegenita veligers delayed metamorphosis an additional 4 d after final onset of competence; this period is almost a third of the planktonic period in this species and may provide enough flexibility to be advantageous to some larvae. Competent larvae of many species of benthic marine invertebrate are known to delay metamorphosis with the proposed advantage of providing larvae with a greater time interval in which to encounter a suitable habitat (Pechenik 1990). Gradual low rates of metamorphosis throughout the delay period are known as "spontaneous" metamorphosis and have been observed in several species maintained in absence of a known inducer (e.g., Highsmith & Emlet 1986). *H. callidegenita* showed a gradual increase in spontaneous metamorphosis throughout the metamorphic period with a peak occurring approximately 5 d after peak time of competence (Figure 3.3) followed by low rates during the remaining planktonic period. Comparison of this peak with the ultimate ability of larvae to delay (4 d after final time of competence) may indicate that larvae are only capable of delaying metamorphosis 4-5 d after competence is reached by an individual larva.

There was no evidence of facultative planktotrophy in *H. callidegenita*, such as uptake of pigment in the digestive gland etc, as has been reported in other lecithotrophic opisthobranchs (Thompson 1958; Kempf & Hadfield 1985). I did not examine the potential of consequences of delayed metamorphosis to the juvenile as has been noted in other species. Such consequences may involve juvenile growth, mortality, and onset of maturity (reviewed by Pechenik 1993). The effect of these consequences may be considerable as veligers are lecithotrophic and therefore cannot replace food reserves throughout the larval period.

Conclusion

In *Haminaea callidegenita*, metamorphic competence is achieved over a two week period in constant presence of inducer, and is independent of larval feeding. All larvae become competent suggesting this pattern may represent a functional extension of the larval

period, rather than reflecting slow development of a few "inferior" larvae. The advantage of this developmental flexibility is twofold. First, competence occurs throughout both the encapsulated and free-swimming phases. Therefore, dispersive propagules and recruits are produced by every individual. Second, dispersing veligers are characterized by 1) an initially high rate of metamorphic competence which rapidly decreases with increasing time hatched, 2) a decrease in larval "choosiness", or an increase in sensitivity to various substrates, and 3) the ability for "spontaneous" metamorphosis. This may be ecologically important in this species because most hatched veligers will experience dispersal over short distances (possibly within or near the large estuarine system inhabited by parents) and few will exhibit longer range dispersal, increasing the potential for gene flow with more distant populations as well as risks associated with a long planktonic period (Palmer & Strathmann 1981; Rumrill 1986). An important component is the variation in time of competence among clutches of larvae. This has not been observed previously and has important implications for both variable development and dispersal potential in this species.

Table 3.1: Induction of metamorphosis before hatching in *Haminaea callidegenita*. A: Results of a one-way ANOVA comparing the % metamorphosis of encapsulated veligers on each of the substrates indicated. B: Scheffé procedure comparing % metamorphosis induced by each substrate with % metamorphosis occuring in seawater only (inducer absent) and in the presence of EMJ (inducer that is present in intact egg masses). *** $p<0.001$, ** $p<0.01$

A. Comparison among all substrates of % metamorphosis/ d

	SOURCE	DF	SS	MS	F	P
Day 1	Between substrates	5	14145.87	2829.17	20.73	***
	Within substrates	77	10511.10	136.51		
	Total	82	24656.97			
Day 2	Between substrates	5	23766.83	4753.37	13.77	***
	Within substrates	77	26563.27	344.98		
	Total	82	50330.11			
Day 3	Between substrates	5	31384.93	6276.98	28.24	***
	Within substrates	77	17113.22	222.25		
	Total	82	48498.15			

B. Comparison of % metamorphosis induced by individual substrates

Substrate	Significant difference from: Seawater			EMJ		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
+ EMJ	**	**	**			
+ 19 mMol K^+	**	**	**	ns	ns	ns
+ Sediment	ns	ns	ns	**	**	**
+ *Chaetamorpha*	ns	ns	ns	ns	**	**
+ *Zostera*	ns	**	**	**	**	**

Table 3.2: Induction of metamorphosis after hatching by EMJ, K^+, *Chaetamorpha linum*, *Zostera marina*, sediment, as well as metamorphosis occuring in seawater only (SW). Data listed are mean (standard errors in lower brackets) percent metamorphosis as a cumulative percent of the total # veligers·substrate·d. n = # cultures/ substrate (10 veligers/ culture).

	SUBSTRATE					
	SW	EMJ	K+	*Chaetamorpha*	*Zostera*	Sediment
N	10	10	10	6	6	6
DAY						
1	0.39 (2.73)	40.17 (9.31)	26.97 (4.83)	0.89 (0.41)	7.46 (5.08)	1.20 (1.78)
2	5.58 (6.05)	56.09 (6.72)	62.35 (6.74)	28.73 (7.98)	15.58 (6.71)	7.43 (6.63)
3	26.04 (6.37)	74.73 (5.13)	71.42 (5.77)	42.09 (9.57)	44.81 (10.97)	28.25 (11.25)
4	46.66 (9.63)	82.93 (5.84)	74.90 (7.36)	86.64 (8.80)	61.04 (8.89)	41.26 (8.66)
5	58.60 (10.29)	85.86 (5.66)	80.48 (5.52)	88.86 (6.84)	74.03 (8.70)	51.26 (7.04)
6	71.91 (8.96)	90.99 (3.83)	84.66 (3.97)	93.32 (4.32)	85.39 (6.47)	68.98 (9.08)
7	76.77 (7.33)	96.19 (2.67)	90.24 (2.62)	96.66 (3.57)	90.26 (3.27)	71.45 (6.63)
8	84.03 (6.25)	97.98 (2.67)	93.03 (2.96)	96.66 (2.65)	93.51 (1.45)	83.55 (5.25)
9	89.59 (6.72)	97.98 (1.58)	94.42 (0.61)	97.77 (0.00)	100 (0.00)	91.66 (2.60)
10	93.35 (1.05)	99.27 (0.00)	100 (0.00)	98.44 (0.00)		92.87 (2.96)
11	94.75 (3.19)	100 (0.00)		100 (0.00)		96.74 (2.16)
12	97.35 (2.89)					98.27 (2.30)
13	98.91 (1.97)					98.27 (0.00)
14	98.92 (0.00)					100 (0.00)
15	100 (0.00)					

Table 3.3: Variable induction of metamorphosis among *Haminaea callidegenita* larvae separated from different egg masses. A: Mean (± standard error) % metamorphosis in response to the substrates indicated at the end of the hatching period. B: Comparison of the % metamorphosis in larvae from different egg masses in response to individual substrates. C: Comparison of inductive potential of EMJ and K+ in terms of response by larvae from different egg masses. Substrates are seawater (inducer absent), EMJ, and 19mmol K^+.
EM= egg mass, EMJ= egg mass jelly.
*** p<0.001, ** p<0.01, * p<0.05

A. % Metamorphosis induced by each substrate

SUBSTRATE	N	X±S.E.	MIN	MAX
Seawater	26	5.65± 1.38	0.00	25.00
EMJ	26	63.35±4.55	12.50	96.30
19 mMol K^+	26	58.23±3.52	30.00	79.74

B. Comparison of the variable response among egg masses (one way ANOVA)

	SOURCE	DF	SS	MS	F	P
Seawater	Between EM	12	0.0595	0.0050	1.0020	ns
	Within EM	13	0.0643	0.0049		
	Total	25	0.1239			
EMJ	Between EM	12	2.3201	0.1933	6.3632	**
	Within EM	13	0.3950	0.0304		
	Total	25	2.7151			
19 mMol K^+	Between EM	12	1.1002	0.0917	5.8037	**
	Within EM	13	0.2054	0.0158		
	Total	25	1.3055			

C. Comparison between EMJ and K^+ as metamorphic inducers (2 way ANOVA)

SOURCE	DF	SS	MS	F	P
EM	12	3.5392	0.2949	11.0905	***
EMJ/ K^+	1	0.2328	0.2328	8.7528	**
Interaction	12	0.7778	0.0266	2.4324	*
Error	30	0.7978	0.0266		

Figure 3.1. Effects of various metamorphic inducers before hatching in *Haminaea callidegenita*. Data are cumulative means (±S.E.) for each day of the hatching period (3 d). n = 15 cultures per substrate (n = 8 for *Chaetamorpha*); each culture contains 20 larvae.

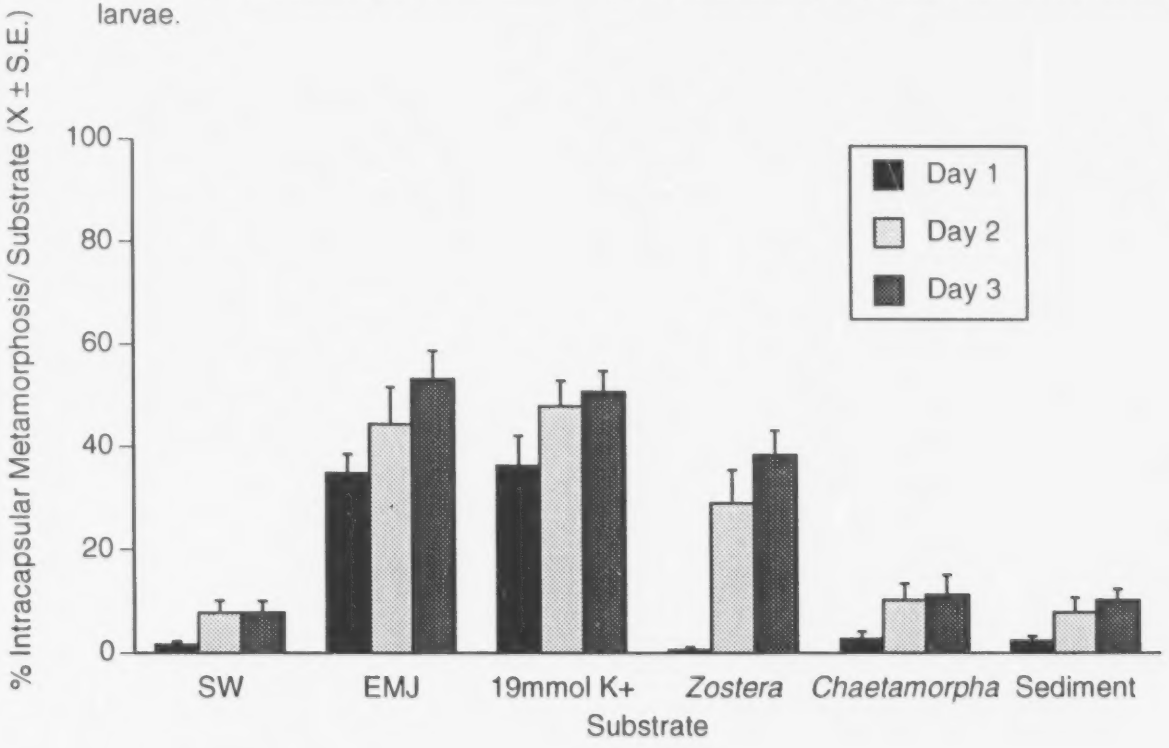


Figure 3.2. Rates of metamorphosis throughout the metamorphic period in *Haminaea callidegenita*. Data are cumulative means per substrate for 10 veligers in each of 6-10 replicates for each substrate.

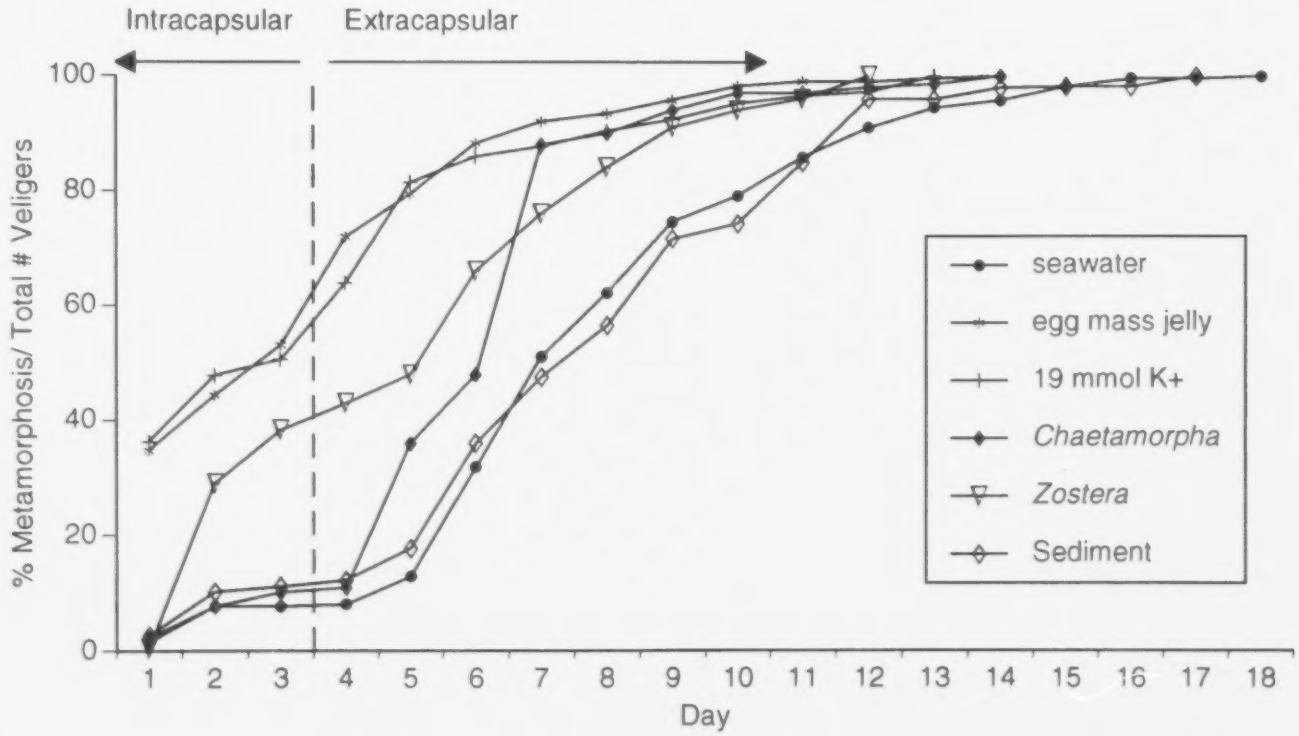
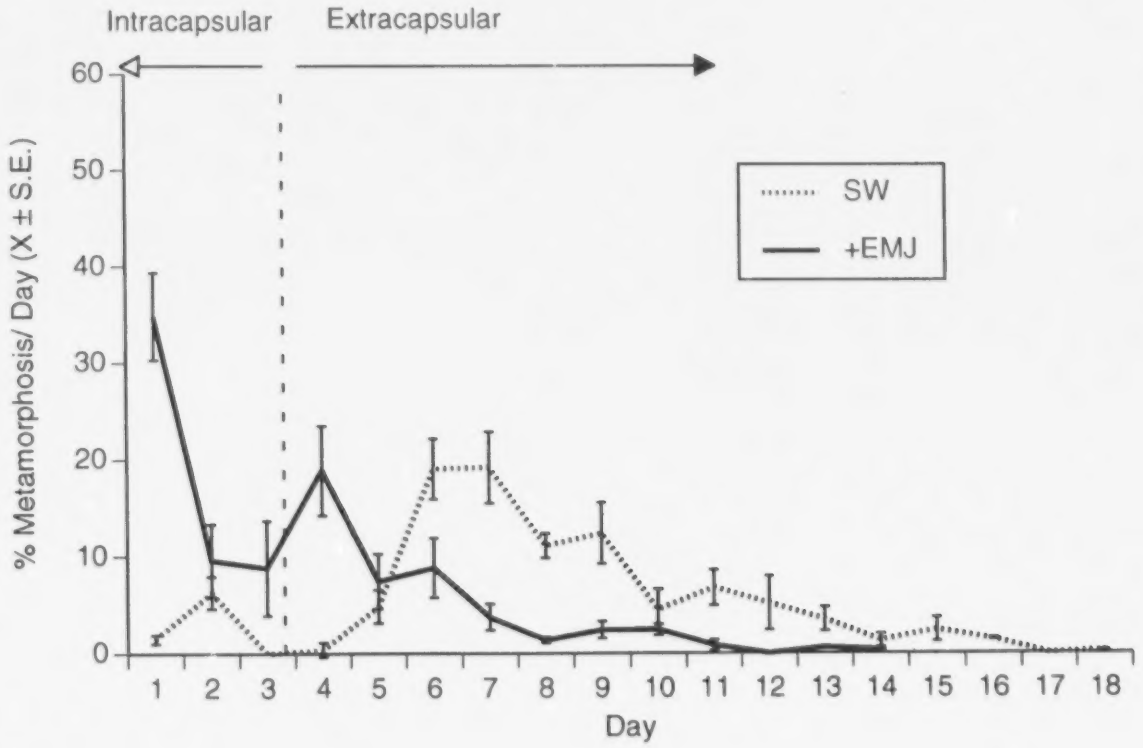


Figure 3.3. Daily rates of metamorphosis of *Haminaea callidegenita* veligers cultured with metamorphic inducer (egg mass jelly; EMJ) or in the absence of inducer (seawater; SW). N = 10 cultures for each group.



Literature Cited

Arkett, S. A., F. S. Chia, J. I. Goldberg and R. Koss. 1989. Identified settlement receptor cells in a nudibranch respond to specific cue. *Biol. Bull. (Woods Hole, Mass.)* **176**:155-160.

Bayne, B. L. 1965. Growth and delay of metamorphosis of the larvae of *Mytilus edulis* (L.). *Ophelia* **2**:1-47.

Birkeland, C., F. S. Chia and R. R. Strathmann. 1971. Development, substratum selection, delay of metamorphosis and growth in the seastar, *Mediaster aequalis* Stimpson. *Biol. Bull. (Woods Hole, Mass)* **141**:99-108.

Burke, R. D. 1983. The induction of metamorphosis of marine invertebrate larvae: stimulus and response. *Can. J. Zool.* **61**:1701-1719.

Cassidy, P. M. and G. L. McKeen. 1986. Padilla Bay baseline water quality record. Report to NOAA/ OCRM/ MEMD by Western Washinton Univ., Shannon Point Marine Center. 472pp. Anacortes, Washington. Padilla Bay National Estuarine Research Reserve Reprint Series No. 2, 1990.

Chia, F.-S. and R. Koss. 1988. Induction of settlement and metamorphosis of the veliger larvae of the nudibranch *Onchidoris bilamellata*. *Int. J. Invert. Reprod. Dev.* **14**:53-70.

Clark, K. B. 1975. Nudibranch life cycles in the Northwest Atlantic and their relationship to the ecology of fouling communities. *Helgoländer wissenschaftliche Meeresuntersuchungen* **27**: 28-69.

Coon, S. L., D. B. Bonar, and R. M. Weiner. 1985. Induction of settlement and metamorphosis of the pacific oyster, *Crassostrea gigas* (Thunberg) by L-DOPA and catecholamines. *J. Exp. Mar. Biol. Ecol.* **94**: 211-221.

Coon, S. L., W. K. Fitt and D. B. Bonar. 1990. Competence and delay of metamorphosis in the Pacific oyster *Crassostrea gigas*. *Mar. Biol.* **106**:379-387.

Crisp, D. J. 1974. Factors influencing the settlement of marine invertebrate larvae. *In*: Chemoreception in Marine Organisms. *Ed.*: Grant, P. T. and A. M. Mackie New York: Academic Press. pp. 177-265.

_____. 1988. Reduced discrimination of laboratory-reared cyprids of the barnacle *Balanus amphitrite amphitrite* Darwin, Crustacea Cirripedia, with a description of a common abnormality. *In*: Marine Biodeterioration. *Ed.*: Thompson, M. F., R. Sarojini, and R. Nagabushanam. New Delhi: Oxford and IBH Publ. Co. pp. 409-432.

Day, R. and L. McEdward. 1984. Aspects of the physiology and ecology of pelagic larvae of marine benthic invertebrates. *In*: eds. Marine Plankton Life Cycle Strategies. *Ed.*: K. A. Steidinger and L. M. Walker, Boca Rotan, FL: CRC Press. pp. 93-120.

Gibson, G. D. and F.-S. Chia. 1989. Developmental variability (pelagic and benthic) in *Haminaea callidegenita* (Opisthobranchia: Cephalaspidea) is influenced by egg mass jelly. *Biol. Bull.* **176**:103-110.

Hadfield, M. G. 1977. Chemical interactions in larval settling of a marine gastropod. *In* Marine Natural Products Chemistry Ed.: D. J. Faulkner and W. H. Fenical. pp. 403-413. New York: Plenum Press.

_____. 1978. Metamorphosis in marine molluscan larvae: an analysis of stimulus and response. *In* Settlement and Metamorphosis of Marine Invertebrate Larvae. Ed.: F. S. Chia and M. E. Rice. New York: Elsevier North-Holland. pp. 165-175.

_____. 1980. Habituation in metamorphic induction of larvae of *Phestilla sibogae* (Gastropoda). *Am . Zool.* **20**:955 (abstract).

Hadfield, M. G. and S. E. Miller. 1987. On developmental patterns of opisthobranchs. *Am. Malac. Bull.* **5**:197-214.

Hadfield, M. G. and D. Scheuer. 1985. Evidence for a soluable metamorphic inducer in *Phestilla*: ecological, chemical and biological data. *Bull. Mar. Sci.* **37**: 556-566.

Harrigan, J. F. and D. L. Alkon. 1978. Larval rearing, metamorphosis, growth and reproduction of the eolid *Hermissenda crassicornis* (Eschscholtz, 1831) (Gastopoda: Opisthobranchia). *Biol. Bull. (Woods Hole, Mass)* **154**:430-439.

Highsmith, R. C. and R. B. Emlet. 1986. Delayed metamorphosis: effect on growth and survival of juvenile sand dollars (Echinoidea: Clypeasteroidea). *Bull. Mar. Sci.* **39**:347-361.

Hirata, K. Y. and M. G. Hadfield. 1986. The role of choline in metamorphic induction of *Phestilla* (Gastropoda, Nudibranchia). *Comp. Biochem. Physiol.* **84**C:15-21.

Lima, G. and J. A. Pechenik. 1985. The influence of temperature on growth rate and length of larval life of the gastropod, *Crepidula plana* Say. *J. Exp. Mar. Biol. Ecol.* **90**:55-71.

Jablonski, D. and R. A. Lutz. 1983. Larval ecology of benthic marine invertebrates: paleobiological implications. *Biol. Rev.* **58**:21-89.

Jackson, G. A. and R. R. Strathmann. 1981. Larval mortality from offshore mixing as a link between pre-competent and competent periods of development. *Am. Nat.* **118**:16-26.

Kempf, S. C. 1981. Long-lived larvae of the gastropod *Aplysia juliana*: do they disperse and metamorphose or just slowly fade away? *Mar. Ecol. Progr. Ser.* **6**:61-65.

Kempf, S. C. and M. G. Hadfield. 1985. Planktotrophy by lecithotrophic larvae of a nudibranch, *Phestilla sibogae* (Gastropoda). *Biol. Bull.* **169**: 119-130.

Knight-Jones, E. W. 1953. Laboraoty experiments on gregariousness during settling in *Balanus balanoides* and other barnacles. J. exp. Biol. **30**:584-598.

Palmer, A. R. and R. R. Strathmann. 1981. Scale of dispersal in varying environments and its implications for life histories of marine invertebrates. *Oecologia* 48:308-318.

Paulay, G., L. Boring, and R. R. Strathmann. 1985. Food limited growth and development of larvae: experiments with natural sea water. *J. Exp. Mar. Biol. Ecol.* 93:1-10.

Pawlik, J. R. 1992. Chemical ecology of the settlement of benthic marine invertebrates. *Oceanogr. Mar. Biol. Annu. Rev.* 30:273-335.

Pechenik, J. A. 1984. The relationship between temperature, growth rate, and duration of planktonic life for larvae of the gastropod *Crepidula fornicata* (L.). *J. Exp. Mar. Biol. Ecol.* **74**:241-257.

_____. 1990. Delayed metamorphosis by larvae of benthic marine invertebrates: does it occur? Is there a price to pay? *Ophelia* 32:63-94.

Pechenik, J. A. and L. Eyster. 1989. Influence of delayed metamorphosis on the growth and metabolism of young *Crepidula fornicata* (Gastropoda) juveniles. *Biol. Bull. (Woods Hole, Mass)* **176**:14-24.

Pechenik, J. A., L. Eyster, J. Widdows and B. L. Bayne. 1990. The influence of food concentration and temperature on growth and morphological differentiation of blue mussel, *Mytilus edulis* L. larvae. *J. Exp. Mar. Biol. Ecol.* **136**:47-64.

Pechenik, J. A. and C. Gee. 1993. Onset of metamorphic competence in larvae of the gastropod *Crepidula fornicata* (L.), judged by a natural and an artificial cue. *J. Exp. Mar. Biol. Ecol.* **167**:59-72.

Pechenik, J. A. and W. D. Heyman. 1987. Using KCL to determine size at competence for larvae of the marine gastropod *Crepidula fornicata* (L.). *J. Exp. Mar. Biol. Ecol.* **112**:27-38.

Pechenik, J., D. Rittschof and A. R. Schmidt. 1993. Influence of delayed metamorphosis on survival and growth of juvenile barnacles *Balanus amphitrite*. *Mar. Biol.* 115: 287-294.

Rumrill, S. S. 1986. Natural mortality of marine invertebrate larvae. *Ophelia* **32**(1-2):163-198.

_____. 1989. Substratum selectivity, post-larval growth and survival following extended competence in *Strongylocentrotus droebachiensis*. *Am Zool.* **29**(4): 29A (abstract).

Scheltema, R. 1986. On dispersal and planktonic larvae of benthic marine invertebrates: an eclectic overview and summary of problems. *Bull. Mar. Sci.* **39**:290-322.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry: The principles and Practice of Statistics in Biological Research, 2nd ed. New York: W. H. Freeman and Co. 859pp.

Thompson, T. E. 1958. The natural history, embryology, larval biology and post-larval development of *Adalaria proxima* (Alder & Hancock) (Gastopoda: Opisthobranchia). *Phil. Trans. R. Soc. Lond. Ser. B.* **242**:1-58.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.* **25**: 1-45.

Trapido-Rosenthal, H. G. and D. E. Morse. 1986a. Regulation of receptor-mediated settlement and metamorphosis in larvae of a gastropod mollusc (*Haliotis rufescens*). *Bull. Mar. Sci.* **39**:383-392.

_____. 1986b. Availability of chemosensory receptors is down-regulated by habituation of larvae to a morphogenetic signal. *Proc. Natl. Acad. Sci.* **83**:7658-7662.

Yool, A. J., S. M. Grau, M. G. Hadfield, R. A. Jensen, D. A. Markell and D. E. Morse. 1986. Excess potassium induces larval metamorphosis in four marine invertebrate species. *Biol. Bull.* **170**: 255-266.

Zimmerman, K. M. and J. A. Pechenik. 1991. How do temperature and salinity affect relative rates of growth, morphological differentiation, and time to metamorphic competence in larvae of the marine gastropod *Crepidula plana*? *Biol. Bull.* **180**:372-386.

Chapter 4

Partial purification of metamorphic inducer from egg mass jelly in the opisthobranch *Haminaea callidegenita*.

INTRODUCTION

Larvae of most species of benthic marine invertebrate are induced to metamorphose by a cue that identifies a specific habitat as suitable for juvenile or adult existence (reviewed by Pawlik 1992). The cue, known as a metamorphic inducer, can be chemical or physical and triggers a radical transformation of morphology, physiology, ecology and behavior of the larva to the juvenile stage. Chemical inducers may be water-borne or tactile, and may originate from con-specifics, prey species, or a characteristic micro- or macrobiotic substrate (bacterial film or an algal, invertebrate or vertebrate species; see Pawlik 1992). Despite many attempts to purify inducers, identification has been made for only four species: the hydroid *Coryne* is induced to metamorphose by a chromanol produced by the brown alga *Sargassum* (Kato et al. 1975); the echiuran *Bonellia* is induced by the pigment bonellin produced by con-specifics (Pelter et al. 1978); the scallop *Pecten* is induced by jacaranone in the red alga *Delesseria* (Yvin et al 1985); and, the polychaete *Phragmatopoma* is induced by free fatty acids (Pawlik 1986) and possibly also by the amino acid L-DOPA, both produced by con-specifics (Jensen & Morse 1990). Metamorphic inducers have been characterized for several other species and include a variety of chemical groups (Pawlik 1992).

The opisthobranch mollusc *Haminaea callidegenita* is induced to metamorphose by a compound found in the gelatinous layers composing most of the egg mass (Gibson & Chia 1989a). A unique life history results in which half the embryos/ egg mass metamorphose in response to jelly before hatching as juveniles. The other half hatch as veligers and will metamorphose in response to jelly throughout the 14 d planktonic period (Chapter 3). Therefore, each parent produces both dispersive (lecithotrophic veliger) and non-dispersive (juvenile) offspring. The objectives of this chapter were: 1) to characterize the metamorphic inducer found in egg mass jelly; 2) to describe purification techniques; and 3) to describe biological characteristics of jelly that influence its activity as a metamorphic inducer. The inducer was smaller than 1000 Da in molecular weight, polar, and stable to temperature and acid. Metamorphic inducing activity was specific to *Haminaea* larvae but the compound was not uniquely produced by adults of this species.

MATERIALS AND METHODS

A. Larval culture

Haminaea callidegenita were collected at Padilla Bay or Spencer Spit, and maintained after collection at the University of Washington, Friday Harbor Laboratory, Washington State, U.S.A.. Culture conditions for adults have been described (Chapter 2). Egg masses used in these assays were either collected in the field or within 24 h of natural spawning by animals maintained in the laboratory. Egg masses were maintained at 17 C until hatching occurred (approximately 14 d after oviposition). Warm temperatures were necessary because the natural population occurs in bays with warmer water (mean of 16.5C from June to August) than in a nearby channel of open water (summer mean of 11.3 C in Rosario Strait; Cassidy & McKeen 1986). Eggs maintained in the laboratory at lower temperatures (10-12 C) had very slow development with a high incidence of abnormal development (pers. obs.).

Embryos used in these assays were separated from egg mass jelly (known to induce metamorphosis; Gibson & Chia 1989a) at the gastrula stage. Egg mass jelly (EMJ) was removed with fine forceps and embryos were left in the embryonic capsule. Separated eggs were rinsed twice in 1 µm filtered seawater to release all traces of jelly. Embryos separated from a particular egg mass were cultured together in 100 ml 1 µm filtered seawater at 17 C. Cultures were cleaned twice weekly by pipetting larvae into freshly filtered seawater (17 C) in glass culture jars that had been scrubbed under hot fresh water to remove primary film. Hatching began approximately 14 d after spawning. In these assays, hatching refers to the escape of larva or juvenile from the capsule and is slightly faster (maximum of 2-3 d) than hatching from intact egg masses (requires 4-5d; Chapter 2).

B. Larval assays

Approximately 4 d before hatching, encapsulated larvae were placed in cultures containing the substrate to be tested as a metamorphic inducer. Cultures were maintained in 2 ml Falcon wells containing 1-2 ml of 1 µm filtered seawater (fSW) and a single inducer (defined below) at 17 C. Approximately 15-20 larvae were placed in each well; number of larvae depended on the size of the egg mass used and the number of wells required for the assay. At 24 h intervals throughout the hatching period all hatchlings were counted and removed under a Wild M-5 microscope. Hatchlings were removed daily so that estimates would not include metamorphosis of hatched veligers. Hatchlings were immediately scored as veligers (with ciliated velum) or juveniles (velar cilia lost and velar lobes resorbed; shells retained through the adult stage). The percent juveniles hatching in each

well was determined for each day of the hatching period and is generally presented as a cumulative percent (number metamorphosed/ total number larvae-metamorphic inducer at the end of the hatching period). Percentage data were arcsin transformed before statistical analysis.

Sample size refers to the number of egg masses (= number of clutches) used. Sample size varies among assays and is given in the figure of a particular experiment. Subgroups of larvae from each mass were simultaneously assayed for each treatment/ assay. It was necessary to compare rates of metamorphosis among clutches because larvae from different egg masses are known to show variable rates of intracapsular metamorphosis (ranging from 4 - 100 %; Chapter 2). This may introduce error in interpretation of results if particular clutches have either very low rates of metamorphosis throughout the hatching period, or high levels of background, or spontaneous, metamorphosis.

All assays contained two cultures ("standard controls") in addition to treatments. These cultures were: 1) separated embryos cultured in filtered seawater (fSW) to determine the proportion of "spontaneous" metamorphosis occurring in each egg mass in absence of inducer, and 2) embryos cultured with untreated EMJ (approximately 1 mm^3) to determine the proportion of embryos/ egg mass competent to metamorphose during the assay period.

C. Characterization of the metamorphic inducer in egg mass jelly

Egg mass jelly (EMJ) was collected, drained of seawater, and frozen at either -9 C or -60 C until assayed for ability to induce metamorphosis. Approximately 1 mm^3 of treated or untreated EMJ (wet volume) was added to the appropriate culture. Antibiotics were added to culture water (both controls and treatments) to prevent degradation of EMJ or jelly extract by bacteria (40 mg/ L each of streptomycin sulfate and penicillin G; Kempf & Willows 1977).

General stability

The stability of the metamorphic inducer in EMJ was determined by boiling (20 min, 100 C) or by lyophilizing homogenized EMJ (24 h, -46 C). Stability of inducer in the presence of acid was determined by acidifying an aqueous EMJ homogenate with glacial acetic acid (2 h, 0.1 M). In all cases, treated EMJ was rinsed with distilled water (distilled water), fSW and approximately 1 mm^3 jelly was added to the appropriate culture.

Effects of proteases

EMJ was treated with one of two non-specific proteases to determine if the inducer was proteinaceous.

a. Proteinase K (final concentration 0.1 mg /ml) was added to 5 ml EMJ for 90 min at 18-20 C. Treated EMJ was rinsed with dilute acid to inhibit the protease, distilled water, and several fSW rinses to remove both proteinase and inhibitor.

b. Protease XIV from *Streptomyces griseus* (Sigma P-5147; final concentration 1 mg /ml) was added to 20 ml EMJ in 20 ml fSW at 17-20 C for 12 h, with continual mixing. EMJ was rinsed with 1 l fSW for each of five times, soaked in fSW overnight, and re-rinsed before assay.

Molecular weight

The molecular weight of inducer was estimated with dialysis and molecular weight filters. A methanol extract was used in this assay, rather than EMJ pieces, to prevent clogging of tubing or filter with the large mucoproteins composing much of the gelatinous matrix.

Dialysis.

A methanol (MeOH) extract of EMJ was dialyzed using tubing of three pore sizes: 12-14000, 6-8000, and 3400 Da (molecular weight cut-off). EMJ was lyophilized, sonicated in absolute MeOH (1 min) and extracted twice with a equal volume of MeOH (30 ml) for 1 h. The extract was centrifuged, supernatants pooled and solvent evaporated under nitrogen leaving an oily, yellow residue. The residue was re-suspended in 150 ml fSW by stirring gently for 1 h. Dialysis tubing was rinsed in distilled water for 2 h to remove the glycerol coating, rinsed in fSW, and filled with 40 ml of inducer suspension. Knots were loosely tied at the end of the tubing to prevent leakage. These bags were rinsed in fSW to remove any external contamination, and placed in 150 ml of fSW that was gently aerated for 24 h at 17 C. The assay included testing the inductive activity of: 1) re-suspended EMJ extract in fSW; 2) the dialyate passing through tubing of each pore size; 3) dialysis tubing prepared and soaked in fSW as above in absence of EMJ to test the effects of the tubing; as well as 4) standard controls (embryos cultured in fSW only and with untreated EMJ).

Ultrafiltration.

Amicon diaflo ultrafilters were also used to estimate molecular weight. Lyophilized EMJ (15 ml wet volume) was twice extracted (1 h each) with 25 ml MeOH. The combined extract was centrifuged at 3000 rpm for 5 min, and MeOH evaporated with a hairdryer. The residue was resuspended in distilled water, and 20 ml suspension was

filtered through either a 5000 Da (Amicon YM5) or a 1000 Da (Amicon YM2) ultrafilter at flow rates of 1 ml/ 2.5 min. All solutions were lyophilized and resuspended in 30 ml fSW. The assay tested the activity of: 1) MeOH residue resuspended in distilled water before centrifugation; 2) distilled water suspension before filtration; 3) filtrates; 4) residue from both YM5 and YM2 filters; and 5) standard controls.

D. Extraction and purification procedures

Extraction with organic solvents

Organic solvents were used to extract inducer from egg mass jelly (Pawlik 1986). Lyophilized EMJ (10 ml wet volume) was sequentially extracted with organic solvents of increasing polarity (20 ml each): hexane, diethyl ether, 1:1 diethyl ether: methanol, methanol, and distilled water. Extraction included sonication for 1 min followed by extraction for 1 h with continual gentle agitation. Solvents were evaporated with a hair dryer, the dry residue re-suspended in fSW and 2 ml added to each Falcon well. Individual solvents were also tested (in absence of EMJ) for potential effects by evaporating an equivalent volume of solvent, and resuspending any possible residue in fSW (cf. Pennington & Hadfield 1989).

High Performance Liquid Chromatography (HPLC)

Metamorphic inducer was partitioned from MeOH extract with HPLC (Spectra-Physics) using 2 buffer systems (gradient and isocratic). In these assays, embryos from more than one egg mass were used for each sample because of the large number of larvae required to test all fractions for inductive activity.

Gradient system

Lyophilized EMJ was extracted with hexane to remove non-polar compounds; eluant was discarded and EMJ dried. Residual EMJ was then sonicated in absolute MeOH (1 min) and further extracted with absolute methanol (5 ml wet EMJ/ 10 ml MeOH) for 1 h. The centrifuged (2 min) MeOH eluant was injected (100 µl) into a Alltech RPC18 column (25 cm x 4.6 mm, 10 µm particle size) and partitioned on a buffer gradient from 100 % methanol to 100 % water over 30 min. Fractions were collected at 1 min intervals. All fractions were tested for inductive activity regardless of the presence of a visible peak (uv-vis detector set at 254 nm). Fractions from three injections (a total of 300 µl crude extract) were combined and pooled at 2 min intervals. Solvent was evaporated, and the re-suspended residue (in fSW) added to Falcon wells and assayed as outlined above. The

assay tested the activity of: 1) crude extract (50 µl/ 2 ml fSW); 2) each 2 min interval (corresponding to 50 µl crude extract/ 2ml fSW); and 3) standard controls.

Two fractions were found to contain activity (min 9-10, 17-18). To determine if both peaks represent the same or similar compounds, fractions containing the first peak of activity (min 9-10) were collected for 3 runs (300 µl original crude extract), concentrated in absolute MeOH and re-partitioned at the same gradient conditions. Fractions were collected and assayed as described above.

Isocratic system

In the final step of the purification procedure, the two active peaks (min 9-10, 17-18) were collected for 3 runs (300 µl crude extract), pooled, dissolved in MeOH and subsequently re-run through the same column isocratically in absolute MeOH to remove additional contaminants. Fractions were collected at intervals corresponding to visual peaks on the chromatograph (detected at 254 nm), and both peaks and inter-peak intervals assayed as outlined above.

E. Activity of metamorphic inducer in egg mass jelly

Activity of metamorphic inducer as a factor of EMJ age

EMJ from masses of different ages were tested for inductive activity. EMJ was separated and pooled from 5 egg masses at each of the following developmental stages: gastrula; early veliger (showing cephalopedal rudiment); mid-veliger (statocysts visible, growth of larval shell complete); late veliger (eyes and heart well developed); and egg masses from which hatching was completed. EMJ was frozen for 48 h before it was assayed. Bioassays were conducted in Falcon wells as detailed above. Data were analyzed with a one-way ANOVA using the Scheffé procedure for unplanned comparisons among treatments (Day & Quinn 1989).

Presence of metamorphic inducer in EMJ produced by other opisthobranch species

EMJ of other opisthobranch species was tested for ability to induce metamorphosis in *Haminaea callidegenita* veligers. Embryos were separated from *H. callidegenita* egg masses when they were gastrulae. Subgroups of 20 embryos from each of 6 egg masses were cultured in the presence of EMJ from one of five opisthobranch species. The metamorphic inducer from *H. callidegenita* EMJ was found to be to be soluble in methanol, and therefore a methanol extract of jelly from each species was also tested for metamorphic activity to determine if potential activity was caused by a chemically similar compound to that found in the EMJ of H. *callidegenita*. EMJ was freeze-dried then extracted with

absolute methanol (5 ml jelly in 10 ml methanol) for 1 h. The methanol extract was centrifuged for 1 min in a microcentrifuge. 100 µl of supernatant was placed in a 2 ml Falcon well, solvent evaporated with a hair dryer and residue re-suspended with fSW.

EMJ was collected from 4 opisthobranch species in addition to *Haminaea callidegenita*, including: *H. vesicula*, a morphologically and ecologically similar congener that occurs in the same habitat; *Melanochlamys diomedea*, also a cephalaspidean and also found in the same habitat; *Alderia modesta*, an ascoglossan found in association with the zanthophyte *Vaucheria* sp. located in the high intertidal zone of the same bays as *H. callidegenita*; and *Onchidoris bilamellata*, a nudibranch found intertidally in association with barnacles. Egg masses of the first three species were collected from False Bay and *Onchidoris* egg masses were collected at Friday Harbor Laboratory, both on San Juan Island, Washington State. Embryos of all egg masses were gastrulae at the time of collection and freezing.

Effects of EMJ as a metamorphic inducer on the larvae of other molluscs

Activity of the metamorphic inducer found in *Haminaea callidegenita* EMJ was tested on 5 other species of mollusc. Species include four opisthobranchs (*H. vesicula*, *Melanochlamys diomedea*, *Alderia modesta*, *Onchidoris bilamellata*) and the oyster *Crassostrea gigas*. Planktotrophic veligers of all five species were cultured as by Kempf & Willows (1977) with the exceptions that antibiotics were not generally used. Phytoplankton supplied as food was a 1:1:1 cocktail of *Isochrysis galbana*, *Pavlova lutheri* and *Rhodomonas* sp.

Larvae were identified as metamorphically competent when the propodium was well developed and the mantle was retracted from the shell (Bickell 1978). These larvae were placed in 100 ml jars (n = 10 larvae/ jar) containing fSW and one of three substrates: 1) EMJ; 2) a metamorphosis inducing substrate associated with that species; or 3) 19 mMol K^+ in fSW. Assays for metamorphic activity of EMJ used either pieces of *Haminaea callidegenita* jelly or an evaporated residue of jelly extracted with methanol. To ensure metamorphic competence, larvae were also tested with a substrate considered suitable for each species. Substrates used included diatoms and adult mucus for *H. vesicula* (Gibson & Chia 1989b), sand containing nematodes for *Melanochlamys* (pers. obs.), the alga *Vaucheria* for *Alderia* (Sceleman 1933), the barnacle *Chthamalus dalli* for *Onchidoris* (Chia & Koss 1988), and adult shell for *Crassostrea* (Crisp 1967). All substrates were collected from the adult habitat of each species. Competence was also determined using seawater containing excess potassium (19 mMol K^+; Yool et al 1986) known to induce

metamorphosis in several phyla of marine invertebrates (Todd et al 1991). Percent metamorphosis was determined after 48 h.

RESULTS

A. Induction of metamorphosis by egg mass jelly

Haminaea callidegenita larvae are induced to metamorphose within the embryonic capsule by egg mass jelly (EMJ). The majority of embryos cultured in the absence of EMJ hatched as veligers (X ± S.E. 12 ± 1 % juveniles released at hatching, n = 24 cultures) while those cultured in presence of inducer undergo intracapsular metamorphosis (52 ± 4 % juveniles, n = 24 cultures) at rates expected of intact egg masses (61 % juveniles, n = 288 egg masses; Chapter 2).

The activity of inducer in EMJ was not destroyed by boiling, lyophilizing, or washing EMJ with acid (Figure 4.1). The activity was also unaffected by the action of two general proteases, proteinase K and pronase XIV (Figure 4.2).

B. Isolation of metamorphic inducer

The metamorphic inducer was dialyzable at all three pore sizes tested with no significant differences among tubing pore sizes (Figure 4.3A) indicating that the metamorphic inducer is smaller than 3400d in molecular weight. This was confirmed by estimations achieved with ultrafiltration in which inducer passed through both 5000 Da and 1000 Da ultrafilters (Figure 4.3B). The decrease in activity observed in these two assays probably reflects the use of an aqueous extract of inducer throughout the dialysis and filtration procedures; solubility of inducer in water is generally lower than in MeOH.

The only organic solvent to extract significant amounts of inducer from EMJ was methanol (Figure 4.4). Some inducer was retained by the EMJ as evidenced by subsequent extraction of inducer with distilled water and the small amount of activity remaining in extracted EMJ. Metamorphosis was not induced by residues of any of the organic solvents tested (Figure 4.4). The MeOH extract showed peak absorbance at 254 and a smaller peak at 303 nm, as well as a peak corresponding to the absorbance maxima for pure MeOH at 205 nm.

C. Purification of metamorphic inducer (HPLC)

Gradient system

Metamorphic inducer eluted at 9-10 min (buffer at approximately 70% MeOH) and at 17-18 min (approximately 40% MeOH) after injection (gradient from absolute MeOH to

H2O; Figure 4.5). Fraction 9-10 induced slightly less metamorphosis (60 %) than occured in response to untreated EMJ (70 %) or the crude MeOH extract (93 %). Fraction 17-18 induced metamorphosis at a much lower rate (32 %; Figure 4.5).

Fraction 9-10 was pooled over 3 runs and re-injected to determine if the inducer would elute as one fraction or if the activity would again partition into two fractions. Inducer again eluted at two intervals, 7-8 min and 15-18 min, with some spreading of each peak (Figure 4.6). In this assay, there were also high levels of spontaneous metamorphosis (e.g., 20 % metamorphosis occurred in veligers cultured in absence of inducer).

Despite some spreading of activity and elution at two peaks, this combination of solvent and column was chosen for the elution protocol because alternative procedures (changing the solvent buffer or column packing) were not as successful or as easily repeated. Increasing the buffer polarity (i.e., starting with water as is more usual in reverse phase chromatography; Quinn 1988) caused rapid elution of the inducer (within 3 min of injection) and no partitioning of contaminants. Decreasing initial buffer polarity (with acetonitrile) caused retention of the inducer on the column (eluted 45 min after injection) accompanied by increased spread of activity. Isocratic buffer failed to partition the inducer from crude extarct. A reverse phase packing for the column was selected because it was the most successful and consistent in initial experiments testing packing with Sep Pacs cartridges. Sep Pacs containing a packing of silica or CN as a functional group did not retain activity. NH_2 Sep Pacs did cause separation of inducer from contaminants, although these results were inconsistent.

Isocratic system

The combined active fractions isolated above (min 9-10, 17-18) were re-eluted using isocratic buffer conditions. This resulted in the elution of inducer as a strong single peak 8 min after injection, as well as the partitioning of several contaminants (Figure 4.7).

Peak 8 was accumulated over several runs. The dry residue was a very small amount of a fine, white powder that reacted strongly with vanillin (indicating the presence of steroids, phenols or essential oils) and had a slight reaction with ninhydrin (indicative of primary amines).

D. Activity of metamorphic inducer in EMJ

Activity of inducer as a factor of EMJ age

There were no significant differences in inductive activity of EMJ of different ages (Figure 4.8) indicating that activity of the metamorphic inducer found in EMJ was not influenced by the age of the jelly.

Presence of metamorphic inducer in the EMJ of other opisthobranch species

EMJ from other opisthobranch species induced metamorphosis in *Haminaea callidegenita* veligers (filled bars; Figure 4.9). Veligers treated with a methanol EMJ extract showed a similar response (hatched bars; Figure 4.9) suggesting that the inducer present in the jelly of other species may be chemically similar to that produced by *H. callidegenita.*

Effects of EMJ as a metamorphic inducer on other species of molluscs

EMJ did not induce metamorphosis in any of the other 4 opisthobranch species tested (Figure 4.10). These larvae metamorphosed in response to excess K^+ in seawater as well as to a substrate thought to be a species-specific metamorphic inducer, indicating that they were competent to metamorphose. The oyster *Crassostrea gigas* did metamorphose in response to EMJ pieces but did not respond to EMJ extract (Figure 4.10). As oyster larvae are known to be metamorphically sensitive to primary film (Coon *et al.* 1985) it is probable that these larvae were induced by a compound associated with the jelly itself (bacteria, diatoms) and were independent of the inducing compound known to be active in *H. callidegenita.* Data shown in Figure 4.10 are the percent metamorphosis/ total number larvae tested on each substrate; the variance among cultures was not estimated.

DISCUSSION

Purification of the metamorphic inducer

Haminaea callidegenita veligers are induced to metamorphose by egg mass jelly. The inducer isolated from EMJ is a polar (methanol soluble) compound that is smaller than 1000 Da in molecular weight, non-proteinaceous and stable to temperature and dilute acid.

Partitioning inducer from crude extract with HPLC produced two peaks of activity. Re-injection of a single active peak resulted in the inducer separating again into two active peaks, eluting at approximately the same times as in the initial injection. This suggests that the inducer either racemates under polar conditions (i.e., exists as 2 configurations in equilibrium) or is degrading during the elution procedure. If racemisation is occuring in this elution protocol, it may indicate that the inducer contains a functional group that is a

hemi-acetyl or hemi-ketyl (R. Andersen, pers. com.). The inducer also inconsistently reacted with the NH_2 packing in Sep Pac preparatory cartridges which act through low capacity ion exchange (Waters Chromatography 1991) suggesting that the inducer may be charged under purification conditions. Degradation of the inducer is less likely because the crude extract retains high levels of activity for at least two weeks (9 C).

A single active peak eluted after re-injection of the combined fractions of partially purified inducer. After drying, the purified inducer appeared as a fine white powder. The slight reaction of this powder with ninhydrin may indicate the presence of a primary amine. The strong reaction with vanillin may indicate the presence of sterols; alternatively, the strength of the reaction may indicate the presence of a residual contaminant, such as a polar lipid that may originate from the presence of eggs in the original egg mass extract. To detect possible contaminants, the pure fraction was re-eluted with HPLC and each fraction scanned with a refractive-index detector to detect the presence of all compounds, regardless of specific ultraviolet or visual absorbance. The result was a single, sharp peak suggesting that this fraction may represent pure inducer, although the presence of a contaminant eluting with the inducer can not be eliminated. Attempts to identify the inducer with NMR were not successful because only small amounts of inducer remained after purification.

Isolation of the metamorphic inducer has been extensively studied in two species of opisthobranch, but in neither case has the inducer been identified. The nudibranch *Phestilla sibogae* is induced by a water-borne compound released from its prey, the hard coral *Porites* (Hadfield 1977). This compound is small (200-500 Da), and temperature and pH stable (Hadfield & Scheuer 1985). The nudibranch *Eubranchus doriae* also metamorphoses in response to its prey species, the hydroid *Kirchenpaueria pinnata* (Bahamondes-Rojas 1988). In this case, the inducer is water soluble and contains galactosidic residues. These veligers are also induced by various sugars as long as the hydroxyl groups attached to carbons 3 and 4 are in the *cis* position (Bahamondes-Rojas & Dherbomez 1990).

Biological characteristics of the metamorphic inducer

The metamorphic inducer found in EMJ is also found in the egg masses of other opisthobranch species suggesting it is an integral component of these gelatinous egg masses. Also, the metamorphic inducer is always present in an active form despite the age of the EMJ. It is possible that it is bound to a non-soluble component of the egg mass (such as the mucopolysaccharide matrix) or if gradually diffusing from the egg mass, it is present in large quantities.

Althouth the same or a similar compound is found in the egg masses of other species, its effects as a metamorphic inducer are restricted to this species. Competent veligers of 5 other molluscan species did not metamorphose in response to the metamorphic inducer in EMJ in a partially purified condition. This is not surprising as larvae are often induced to metamorphose by a highly specific substrate characteristic of a suitable juvenile or adult habitat (Pawlik 1992). *Haminaea vesicula* and *Melanochlamys diomedea* coexist with *H. callidegenita* but their larvae metamorphose in response to cues associated with more general characteristic of the habitat.

Figure 4.1. Effect of temperature and acid on inductive activity of *Haminaea callidegenita* egg mass jelly. Data are the percent intracapsular metamorphosis of *H. callidegenita* veligers occurring in response to EMJ that was boiled (B), lyophilized (L), or washed with dilute acid (H+). The first two columns indicate metamorphosis occurring in absence of inducer (SW) and in the presence of untreated EMJ. The horizontal bar indicates that no significant differences were found among the four EMJ groups (one way ANOVA).

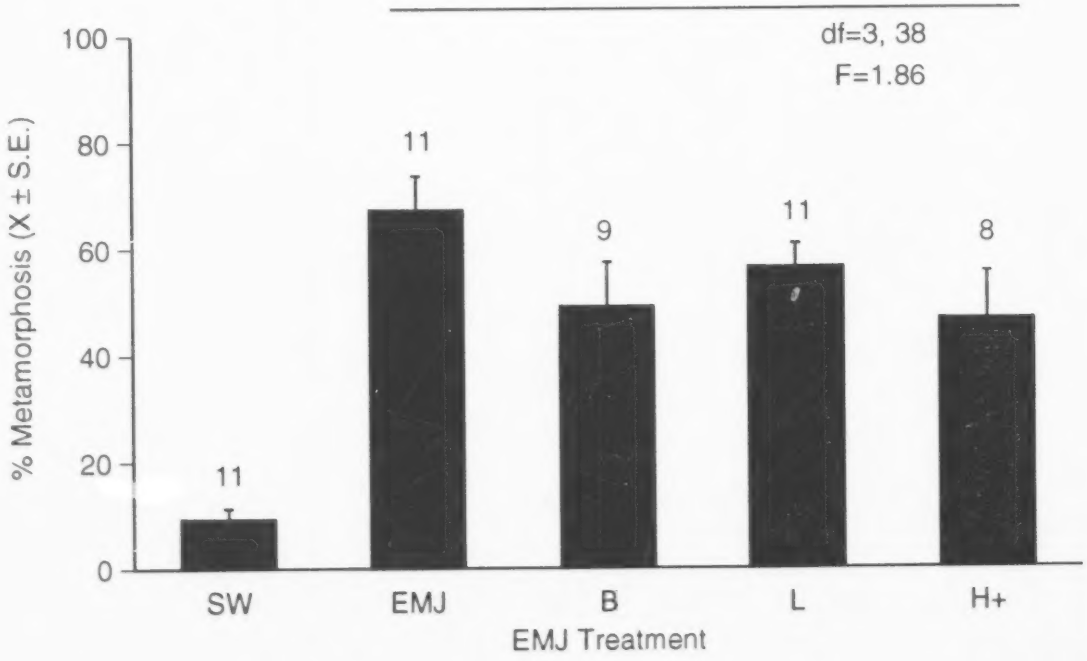


Figure 4.2. Effects of proteases on activity of inducer. Data are the percent intracapsular metamorphosis induced in *Haminaea callidegenita* veligers by untreated EMJ and EMJ treated with proteinase K (solid bars) or pronase XIV (hatched bars). Veligers were also cultured in absence of inducer (SW). Horizontal bars indicate no significant differences were found between treated and untreated EMJ (unpaired two-tailed t-test).

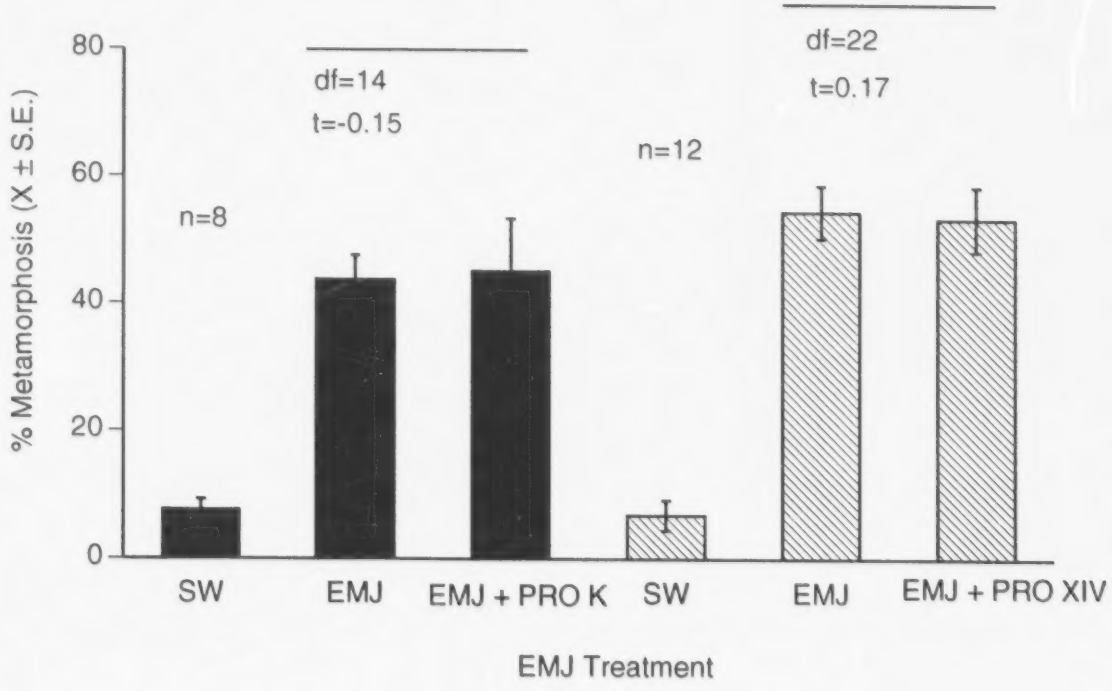


Figure 4.3. Estimation of inducer molecular weight. A. Effects of dialysis. Data are the percent intracapsular metamorphosis of *Haminaea callidegenita* veligers cultured in seawater only (SW), with untreated inducer (EMJ), with residue of a crude jelly extract (EXTRACT),with dialyate from the indicated pore sizes (Da), and with dialysis bag only. B. Effects of ultrafiltration. The methanol extract was re-suspended (SUSP), centrifuged (both supernatant C-S) and pellet C-P tested), and filtered.

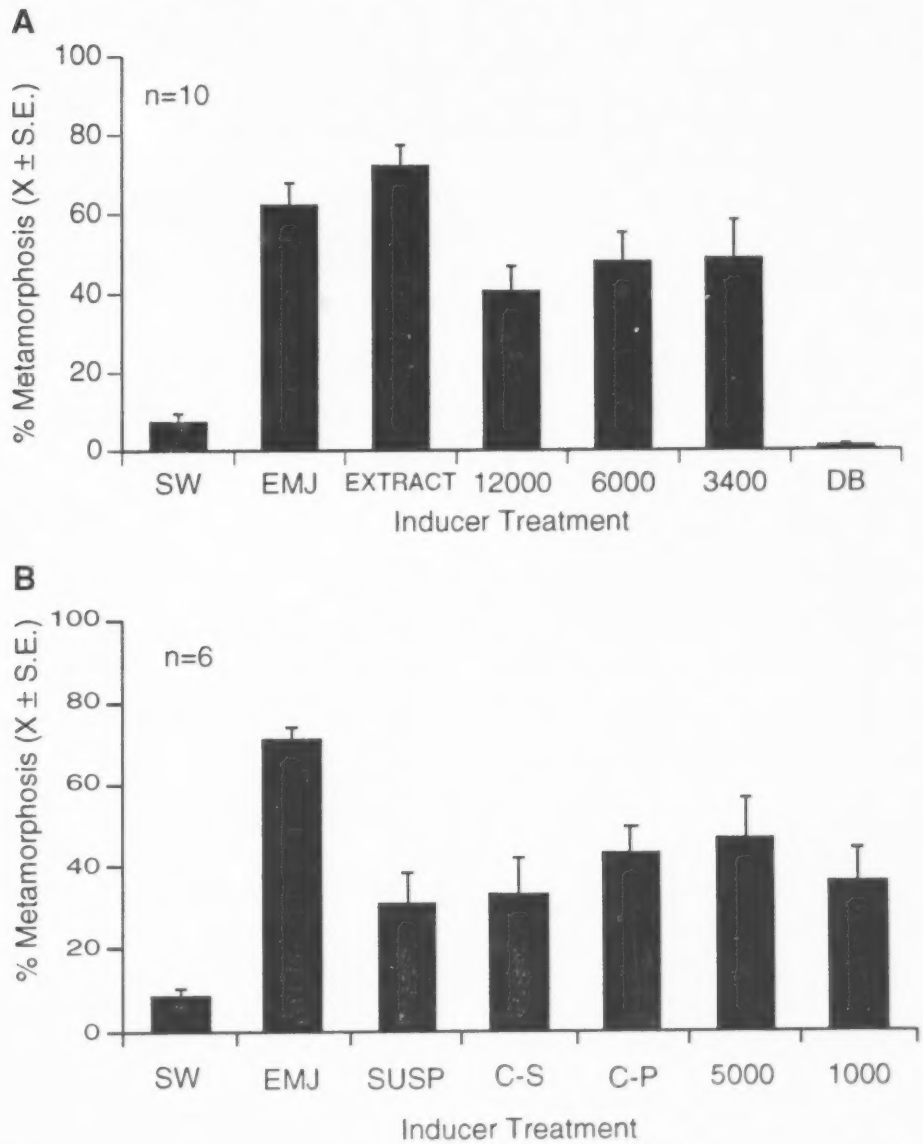


Figure 4.4. Extraction of metamorphic inducer with organic solvents. Data are the percent intracapsular metamorphosis in *Haminaea callidegenita* veligers cultured in seawater (SW), with untreated inducer (EMJ), with EMJ extracts from hexane, diethyl ether, 1:1 ether: methanol (E:M), methanol, water, and with EMJ residue after extraction (solid bars). The effects of solvent residue after evaporation were also tested for inductive activity (hatched bars).

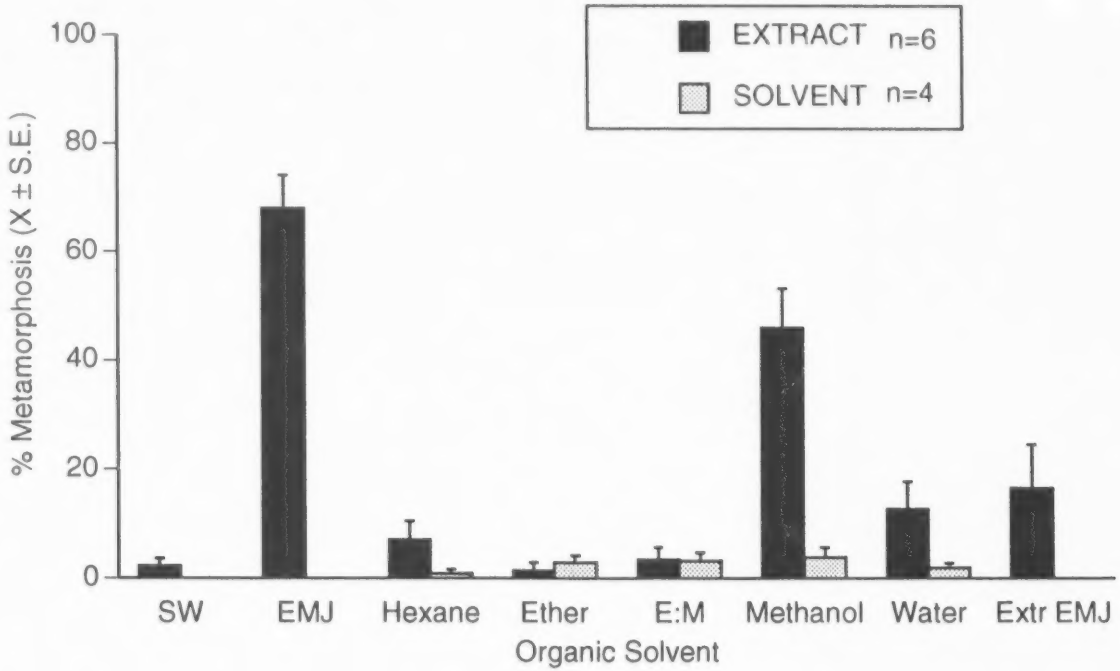


Figure 4.5. Isolation of inducer with High Performance Liquid Chromatography (buffer gradient). A. Chromatograph of crude EMJ extract on a RP C18 column, detected at 254 nm. Fractions were collected at 2 min intervals (total of 30 min). B. Percent intracapsular metamorphosis in seawater (SW), with untreated inducer (EMJ), with crude EMJ extract (EXTR), and with the indicated fractions.

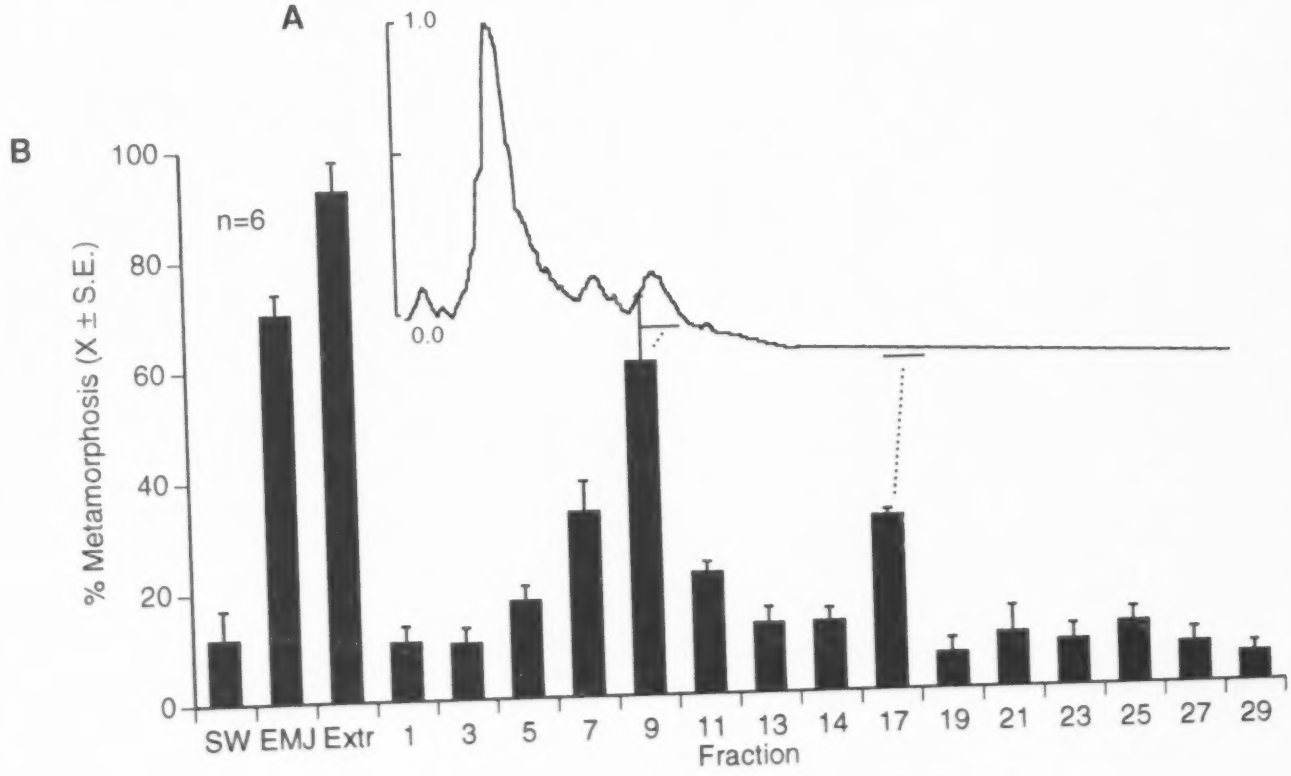


Figure 4.6. Re-isolation of first active fraction (min 9-10) with High Performance Liquid Chromatography (buffer gradient). A. Chromatograph of re-injected fraction 9-10 on a RP C-18 column, at 254 nm. Fractions were collected at 2 min intervals (total 30 min). B. Percent intracapsular metamorphosis of veligers in seawater (SW), with untreated EMJ, with a crude jelly extract, with fraction 9-10, and with the indicated fractions after elution.

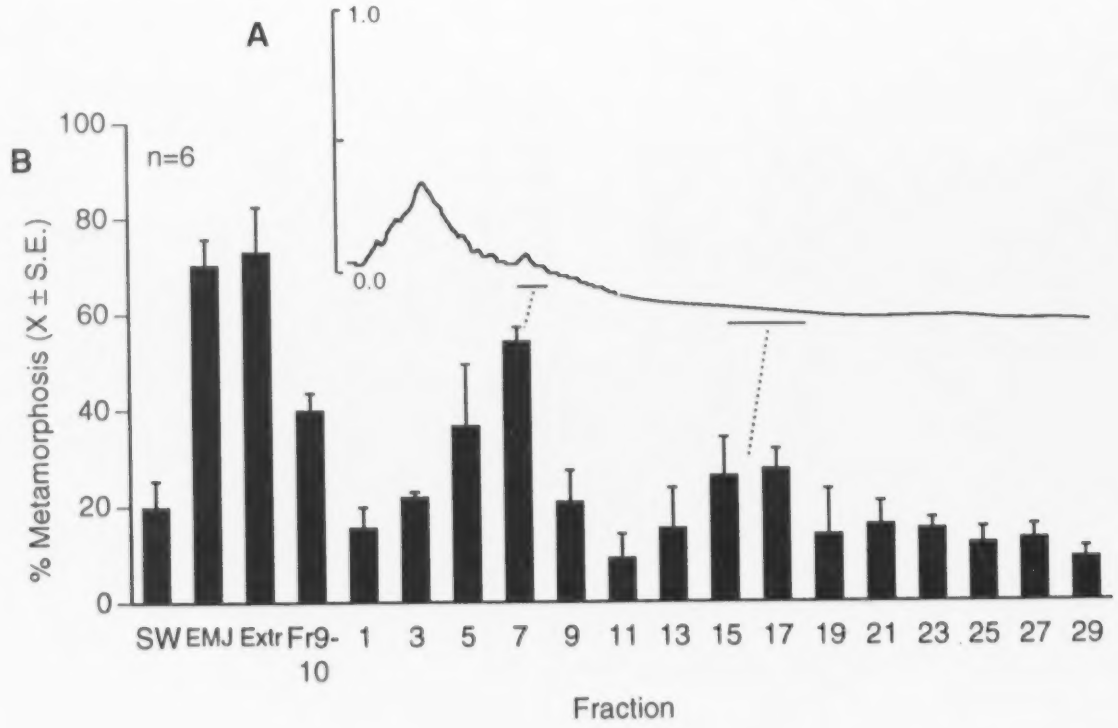


Figure 4.7. Purification of inducer from isolated active fractions with High Performance Liquid Chromatography (isocratic gradient). A. Chromatograph of in re-injected active fractions (min 9-10, 17-18) on a RP C-18 column, detected at 254 nm. B. Percent intracapsular metamorphosis occurring in response to seawater (SW), untreated inducer (EMJ), and the eluted fractions as indicated.

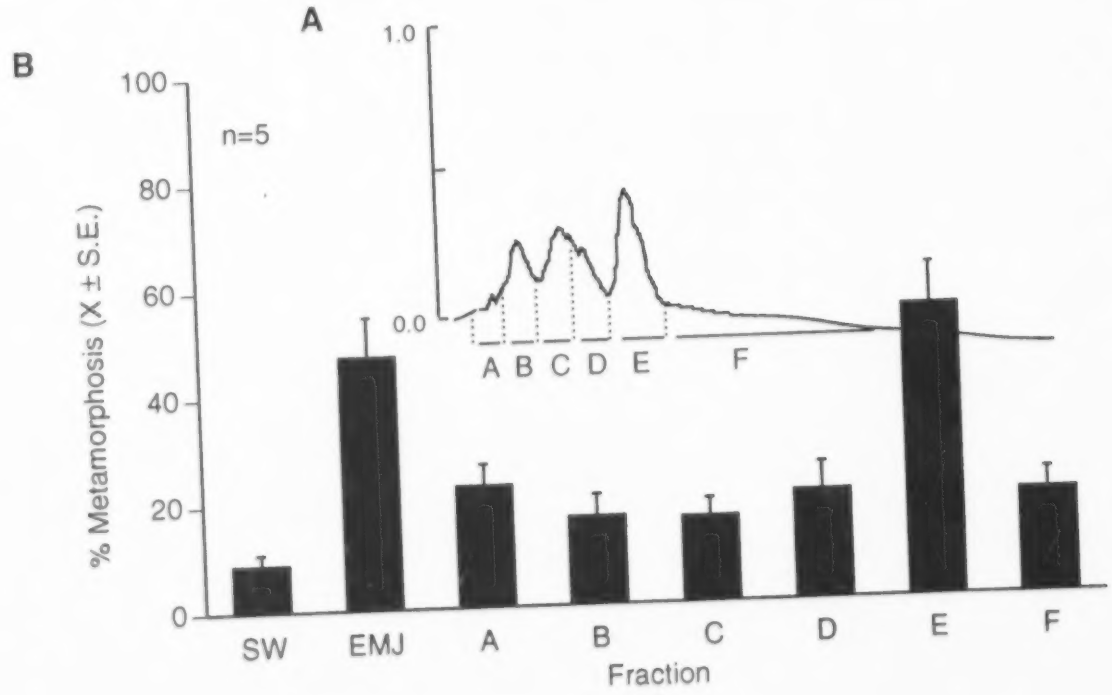


Figure 4.8. Effect of jelly age on inductive activity. Data are percent juveniles released at hatching for embryos cultured in seawater (SW) and with EMJ from egg masses at the stage indicated. The horizontal bar indicates no significant differences were found among these groups (1 way ANOVA).

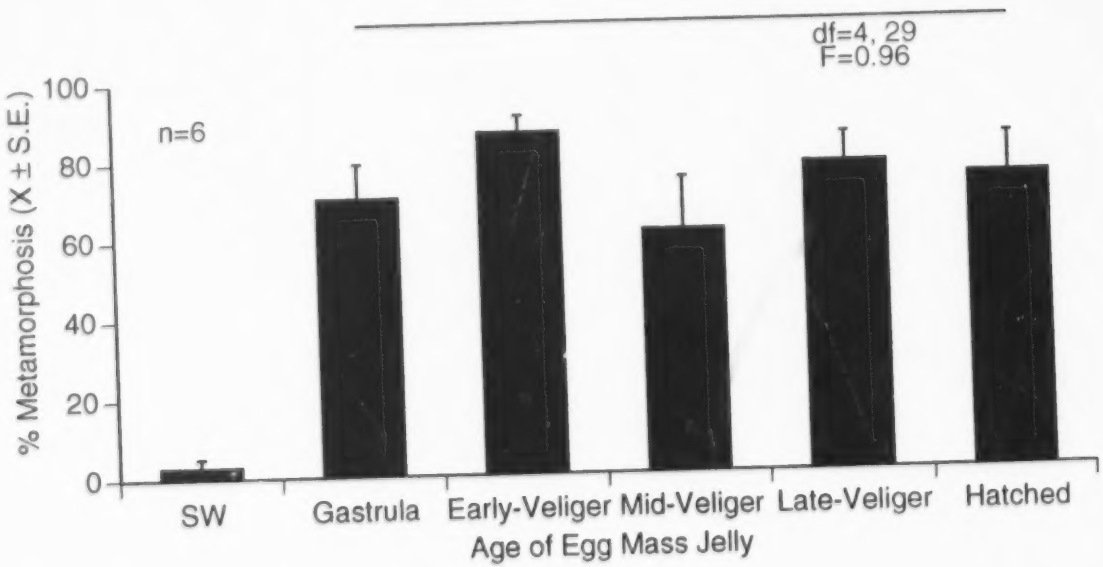


Figure 5.9. Metamorphic inducing activity in the EMJ produced by other species. Data are the percent intracapsular metamorphosis in *Haminaea callidegenita* veligers in seawater (SW), with EMJ pieces (solid bars) or with EMJ extract (hatched bars). EMJ was produced by Hc = *H. callidegenita*, Hv = *H. vesicula*, Am = *Alderia modesta*, Ob = *Onchidoris bilamellata*, and Md = *Melanochlamys diomedea*.

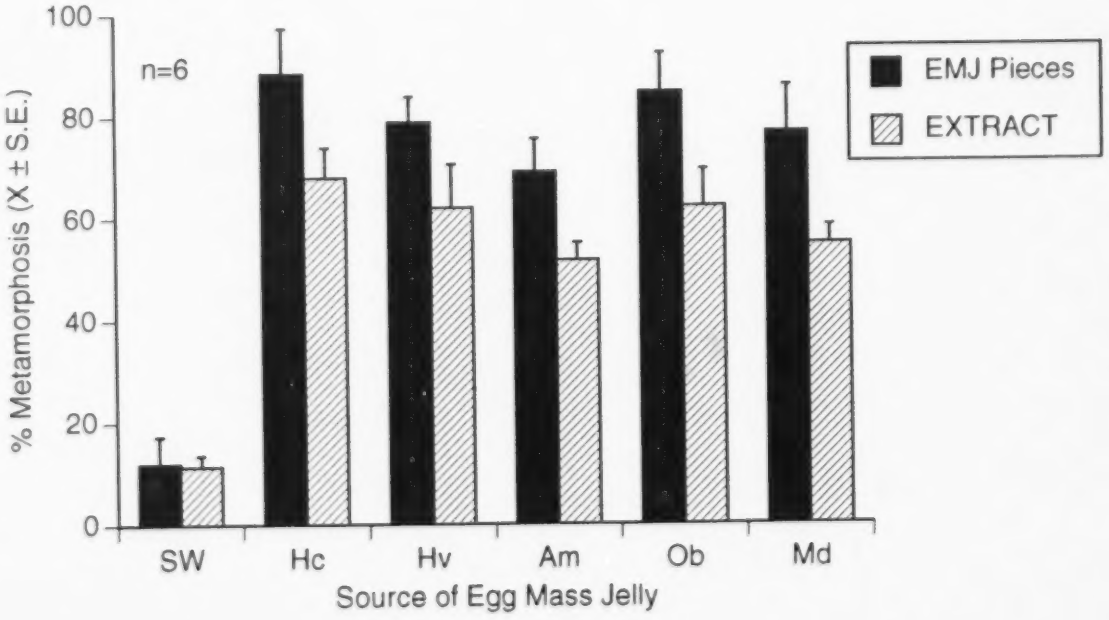
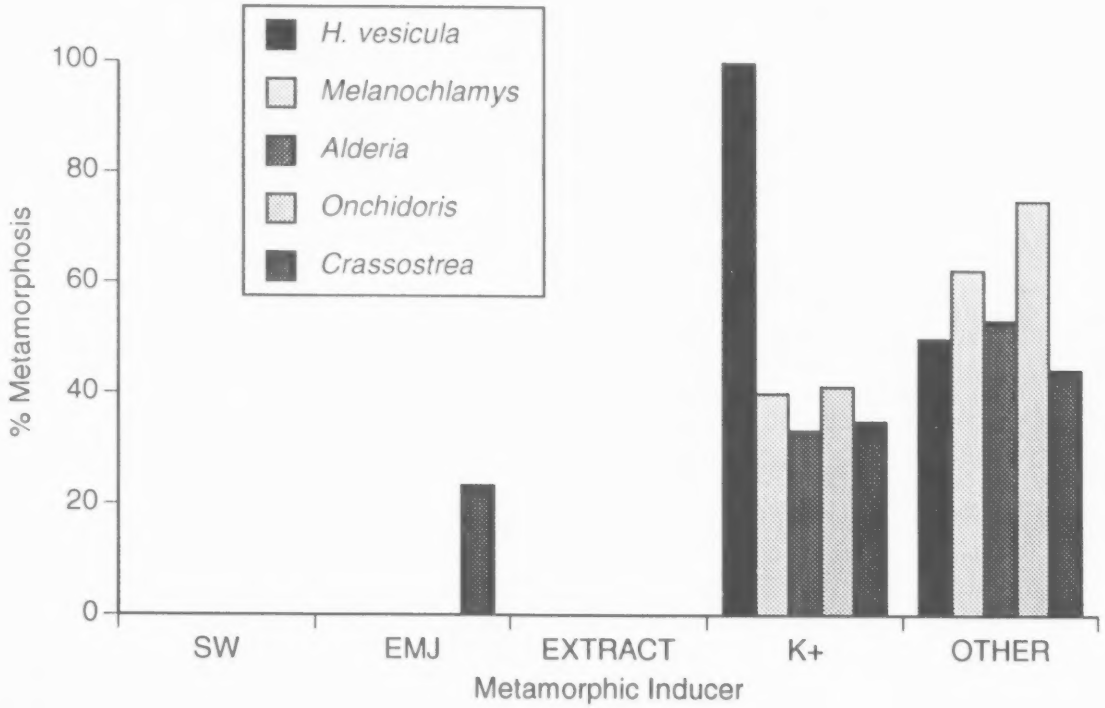


Figure 4.10. Induction of metamorphosis in 5 molluscs by *Haminaea callidegenita* EMJ, a jelly extract (EXTRACT), 19 mmol K+, and a substrate associated with metamorphosis in that species (see text).



Literature Cited

Bahamondes, I. 1988. Induction de la métamorphose des larves d'*Eubranchus doriae* (Trinchèse, 1879), Mollusque Nudibranche, pae des substances provenant de l'espèce proie *Kirchenpaueria pinnata*, Hydrozoaire, Cnidare. *Vie Mar.* **9**:1-6.

Bahamondes-Rojas, I. and M. Dherbomez. 1990. Purification partielle de substances glycoconjuées capables d'induire la metamorphose des larves compétentes d'*Eubranchus doriae* (Trinchèse, 1879), mollusque nudibranche. *J. Exp. Mar. Biol. Ecol.* **144**:17-27.

Bickell, L. R. 1978. Larval Development, Metamorphosis and Juvenile Feeding of *Doridella steinbergae* (Lance) (Opisthobranchia: Nudibranchia). M. Sc. Thesis, University of Alberta. 226 pp.

Cassidy, P. M. and G. L. McKeen. 1986. *Padilla Bay baseline water quality record.* Report to NOAA/ OCRM/ MEMD by Western Wash. Univ., Shannon Point Marine Center. Anacortes, Washington: Padilla Bay National Estuarine Reserach Reserve Reprint Series No. 2, 1990. 472pp.

Chia, F.-S. and R. Koss. 1988. Induction of settlement and metamorphosis of the veliger larvae of the nudibranch, *Onchidoris bilamellata*. *Int. J. Invert. Reprod. Dev.* **14**:53-70.

Coon, S. L., D. B. Bonar and R. M. Weiner. 1985. Induction of settlement and metamorphosis of the Pacific oyster, *Crassostrea gigas* (Thunberg) by L-DOPA and catecholamines. *J. Exp. Mar. Biol. Ecol.* **94**:211-221.

Crisp, D. J. 1967. Chemical factors inducing settlement in *Crassotrea virginica* (Gmelin). *J. Anim. Ecol.* **36**:329-335.

Day, R. W. and G. P. Quinn. 1989. Comparisons of treatments after an analysis of variance in ecology. *Ecological Monographs* **59**(4):433-463.

Gibson, G. D. and F.-S. Chia. 1989a. Developmental variability (pelagic and benthic) in *Haminaea callidegenita* (Opisthobranchia: Cephalaspidea) is influenced by egg mass jelly. Biol. Bull. **176**:103-110.

_____. 1989b. Embryology and larval development in *Haminaea vesicula* Gould (Opisthibranchia: Cephalaspidea). *Veliger* **32**:409-412.

Jensen , R. A. and D. E. Morse. 1990. Chemically induced metamorphosis of polychaete larvae in both the laboratory and ocean environment. *J. Chem. Ecol.* **16**:911-930.

Kato, T., A. A. Kumanireng, I. Ichinose, Y. Kitahara, Y. Kakinuma, M. Nishihira, and M. Kato. 1975. Active components of *Sargassum tortile* effecting the settlement of swimming larvae of *Coryne uchidai*. *Experimentia* **31**:433-434.

Kempf, S. C. and A. O. D. Willows. 1977. Laboratory culture of the nudibranch *Tritonia diomedea* Bergh (Tritoniidae: Opisthobranchia) and some aspects of its behavioral development. *J. exp. mar. Biol. Ecol.* **30**:261-276.

Pawlik, J. R. 1986. Chemical induction of larval settlement and metamorphosis in the reef-building tube worm *Phragmatopoma californica* (Polychaeta: Sabellariidae). *Mar. Biol.* **91**: 59-68.

Pawlik, J. R. 1992. Chemical ecology of the settlement of benthic marine invertebrates. *Oceanogr. Mar. Biol. Annu. Rev.* **30**:273-335.

Pelter, A. J. A. Ballantine, P. Murray-Rust, V. Ferrito and A. F. Psaila. 1978. The structures of anhydrobonellin and bonellin, the physiologically active pigment from the marine echiuroid *Bonellin viridis*. *Tetrahedron Lett.* **21**:1881-1884.

Pennington, T. and M. G. Hadfield. 1989. Larvae of a nudibranch mollusc (*Phestilla sibogae*) metamorphose when exposed to common organic solvents. *Biol. Bull. (Woods Hole, Mass.)* **169**:417-430.

Quinn, R. J. 1988. Chemistry of Aqueous Marine Extracts: Isolation Techniques. *In* Bioorganic Marine Chemistry Vol. 2 *Ed.*: P. J. Scheuer. Berlin: Springer-Verlag. pp. 1-41.

Seeleman, U. 1933. Rearing experiments on the amphibian slug *Alderia modesta*. *Helgo. wiss. Meeresunters* **15**(1):128-134.

Todd, C. D., M. G. Bentley, and J. N. Havenhand. 1991. Larval metamorphosis of the opisthobranch mollusc *Adalaria proxima* (Gastropoda: Nudibranchia): the effects of choline and elevated potassium ion concentration. *J. Mar. Biol. Assoc. U. K.* **71**:53-72.

Yool, A. J., S. M. Grau, M. G. Hadfield, R. A. Jensen, D. A. Markell and D. E. Morse. 1986. Excess potassium induces larval metamorphosis of marine marine invertebrate species. *Biol. Bull. (Woods Hole, Mass.)* **170**:255-266.

Yvin, J. C., L. Chevolot, A. M. Chevolot-Magueur and J. C. Cochard. 1985. First isolation of jacaranone from an alga, *Delesseria sanguinea*, a metamorphosis inducer of *Pecten* larvae. *J. Nat. Prod.* **48**:814-816.

Personal Communications:

Dr. Raymond Andersen,
Oceanography Department,
University of British Columbia,
Vancouver, British Columbia.

Chapter 5

Dispersal polymorphism in *Haminaea callidegenita* larvae (Mollusca: Opisthobranchia) originates from genetic and environmental factors.

INTRODUCTION

Benthic marine invertebrates show one of three life history patterns that are defined by larval type (planktotrophy, lecithotrophy, and direct development; Thorson 1950). Each pattern is characterized by a suite of phenotypic traits including parental reproductive traits (e.g., egg size and fecundity) and larval characteristics (e.g., duration of the planktonic period and ability to feed). The evolutionary implications of each mode have been much discussed with emphasis on the role of the planktonic larva (Mileikovsky 1971; Vance 1973 a&b; Caswell 1983; Strathmann 1985, 1986). The planktonic larva is the primary dispersal phase for most species and therefore has a major influence on population dynamics (colonization, recruitment, and growth; Day & McEdward 1984), population genetics (gene flow among existing populations; Scheltema 1986), and speciation and extinction events (Jablonski & Lutz 1983).

Understanding the role these traits play in life history evolution requires an examination of the trait on several levels including phenotypic variability, heritability, and genetic correlation between traits (Roff 1992). Selection acts on phenotypic variability but unless these results are passed on to the next generation, evolutionary change will not occur. Phenotypic descriptions and comparisons of reproductive traits are common and create the foundation for life history theory in benthic marine invertebrates (see summary by Strathmann 1986). However, heritabilities and genetic correlations for life history traits have been largely ignored. These data are necessary to predict effects of selection on individual traits and of trade-offs and constraints among traits. An exception is the work of Levin *et al.* (1991) who examined the genetic basis life history traits in the polychaete *Streblospio benedicti* , a species with variable development in which individuals produce either planktotrophic (feeding larvae with a long planktonic period) or lecithotrophic offspring (non-feeding larvae with a shorter planktonic period). In this species, length of the swimming phase is genetically determined while larval feeding ability is not.

Marine invertebrate species that have variable development are ideal for examining life history traits because observations are free of the complications arising from multiple species comparisons; i.e., observed differences may be caused by morphology, physiology, or phylogeny rather than development mode. The focus of the present study

was another species with variable development, in this case based on dispersal potential of offspring. In the mollusc *Haminaea callidegenita* (Opisthobranchia: Cephalaspidea), both swimming, non-feeding larvae (veligers) and juveniles are released from every egg mass (Gibson & Chia 1989). The consequence of this developmental pattern is that every individual produces two types of offspring: one providing a high potential for dispersal and one allowing immediate recruitment into the parental habitat. All embryos/ egg mass simultaneously pass through the same developmental stages and the difference in hatchling type arises from time of metamorphosis. At hatching half of the embryos/ egg mass hatch as veligers, while the other half metamorphose within the embryonic capsule and hatch as juveniles. Metamorphosis is induced in siblings undergoing intracapsular metamorphosis by a compound present in the jelly layers covering the egg mass (Gibson & Chia 1989).

My objective in this study was to determine if several life history traits were heritable in *Haminaea callidegenita*. Traits included: 1) reproductive traits (e.g., embryonic period, hatching period); 2) time of metamorphosis of offspring (before or after hatching), which determine offspring type and dispersal potential; and 3) ability of larvae to delay metamorphosis. I also examined the role of parental environment in determining time of metamorphosis and therefore offspring type. Correlations among these traits were examined in Chapter 6.

MATERIALS AND METHODS

A. Animal collection and culture

Haminaea callidegenita were collected from Padilla Bay (48°30'N, 122°29'W) and maintained at the University of Washington, Friday Harbor Laboratory, both in Washington, U. S. A. (Figure 5.1). Padilla Bay is at the head of a large estuarine system and is dominated by extensive mud flats and seagrass beds composed of both *Zostera marina* and *Z. japonica*. *H. callidegenita* adults and egg masses were collected from *Z. japonica* beds in the upper intertidal zone. Adults and juveniles graze diatoms and detritus from the grass blades as well as ingesting *Ulva* sp. (sea lettuce). *H. callidegenita* are simultaneous hermaphrodites and store allosperm for up to 7 months (pers. obs.). They grow to approximately 30 mm during their annual lifespan. Oviposition begins at approximately
16 mm, but copulation begins in animals of 10 mm length, and allosperm may be stored throughout the intervening period (pers. obs.).

To ensure known parentage of egg masses, juvenile *Haminaea callidegenita* were collected (< 8 mm in length; in October 1991 and September 1992) and maintained in

isolation until reproductively mature (July 1991 and April 1992). Juveniles were maintained in polypropylene cages lined with 224 µm mesh that were approximately 75 ml in volume (Toby Teaboys ™, Aldridge Plastics Ltd.). Cages were held in outdoor tanks with flowing seawater at ambient conditions (in summer, 9-12 C). *Ulva* sp. was continuously supplied as food. *Ulva* was replaced at two week intervals with sea lettuce collected intertidally at Friday Harbor Laboratory. *Zostera* was not provided as food because of its high laboratory mortality. Animals also grazed diatoms and detritus that accumulated on the inside of the cage walls.

As juveniles grew, they were transferred to larger cages, 1 L Tripour beakers with two 70 mm^2 500 µm Nitex mesh vents to allow continual seawater flow. Food was supplied as above. *H. callidegenita* were considered mature when approximately 16 mm in length, at which time a few animals began to produce egg masses. These initial egg masses were not viable and development stopped after the first few cleavage stages, indicating that the eggs had not been fertilized and therefore the experimental animals had neither mated before the onset of the experiment nor had self fertilized. Size was determined as body length measured while the animal was actively crawling (mm) and as blotted wet weight (mg).

Cages were checked daily for the presence of freshly spawned egg masses. These egg masses were removed and cultured in glass jars containing 50 ml of 1 µm filtered seawater at 17 C. The warm temperature of the culture water was similar to summer temperatures in Padilla Bay (mean of 16.5 C; Cassidy & McKeen 1986). Culture water was replaced and jars cleaned with hot water twice weekly throughout the experimental period. Throughout the hatching period egg masses were examined at 24 h intervals to determine several life history traits (defined below). Females spawn repeatedly over several weeks and all egg masses spawned by each female were collected (between 6-7 egg masses for most females, ranging from 1 to 14 overall). Most egg masses showed negligible, if any, embryo/ larval mortality. Egg masses with >2-3% mortality were discarded from the experiment.

B. Genetic analysis

Breeding design and definition of traits

H. callidegenita were mated in a hierarchical design allowing sib-analysis (Falconer 1960), also known as North Carolina Design 1 (Lawrence 1984), the most powerful design possible given the reproductive biology of the animals. Measurement of progeny with this breeding design gives estimates for half-sib (through sire components) and full-

sib families (dam components). Eight family groups were maintained in 1991 and 11 groups in 1992.

The design consists of groups composed of one "male" bred to each of four "females" (Figure 5.2). *Haminaea* are simultaneous hermaphrodites and are capable of storing sperm for several months of their annual lifespan, thus preventing a reciprocal mating design. Designation of "male" or "female" was arbitrary and all egg masses produced by "males" were discarded from the experiment. To ensure known paternity, males were paired individually with each female by placing the male (e.g., male A) in the cage of a particular female for 24 h (female A1), removing the male and placing him in the cage of the second female (A2) for 24 h and so on (for females A1 through A4), then returning the male to his original cage. Mating was repeated at 2 week intervals. Differences in dorsal pigmentation made identification of paired individuals straightforward and prevented accidental mixing of individuals.

I examined several life history traits including female reproductive characteristics and time of metamorphosis in offspring. Each trait was treated as a quantitative character, or as one showing continuous variation and probably determined by the combined action of several genes. Although time of metamorphosis is expressed phenotypically as an all-or-none response, it was considered here as a quantitative character. All-or-none traits are often analyzed as quantitative traits with the assumption that they represent a threshold character imposing discontinuity on an underlying continuously varying trait (Wright 1933; MacKay & Doyle 1978). One egg mass (or one clutch of eggs) is considered to represent one progeny in this analysis. Each trait was measured on every egg mass produced with the exception of time of metamorphosis of hatched veligers where I was limited by the number of hatched larvae released/ egg mass. Traits are defined in Table 5.1 and include reproductive traits (1991 and 1992) and time of metamorphosis of offspring, both within the egg mass (both years) and after hatching (1992 only).

The number of hatchlings per egg mass was determined by counting hatched veligers and juveniles daily throughout the hatching period then calculating the total per mass. Embryo mortality is very low in *H. callidegenita*; a few egg masses showed a high embryo mortality (>2 %) and these were discarded from the analysis.

Time of metamorphosis was determined for both the pre-hatching and post-hatching periods. The percent of juvenile hatchlings was determined by removing hatchlings daily throughout the hatching period. Hatchlings were immediately scored as veligers or juveniles to give the percentage of total hatchlings that hatched as juveniles; this value was arcsin transformed prior to statistical analysis (Sokal & Rohlf 1981). Veligers were identified by the presence of an intact velum and were either swimming or crawling when

counted. Juveniles had lost velar cilia and velar lobes were resorbed. Estimates of time of metamorphosis may have been inflated by the metamorphosis of hatched veligers within the 24 h intervals between counts; however, this effect was judged to be slight as the mean time of the first metamorphosis/ clutch after hatching was approximately 3 d post-hatching.

Time of metamorphosis after hatching was measured by culturing hatched veligers continuously in the presence of metamorphic inducer (egg mass jelly; EMJ), and counting newly metamorphosed juveniles at 24 h intervals. Juveniles were removed after metamorphosis. Up to 30 hatched veligers/ egg mass were placed in 2 ml Falcon wells with up to 5 veligers/ well; number of veligers depends on number hatched from each egg mass. Wells contained 1 µm filtered seawater and approximately 1 mm^3 EMJ. Culture water and jelly were replaced twice weekly and cultures were maintained until all veligers had metamorphosed. The planktonic period was separated into 3 intervals: minimum planktonic period/ clutch, median time of metamorphic competence, and maximum time of competence (Table 5.1).

The ability of larvae to delay metamorphosis was determined by culturing hatched veligers as above, only in absence of metamorphic inducer. Sea water was filtered and sterilized (microwaved at high power for 3 min, water boiling for at least 1 min) to prevent contamination from bacteria and diatoms and the possible inadvertent induction of metamorphosis by other sources (Chapter 3). The planktonic period was separated into 3 intervals as outlined above (Table 5.1).

The effects of egg size on time of metamorphosis were also considered. Egg size was excluded from the overall genetic comparison because eggs were measured in only a few egg masses produced/ female. Maximum and minimum dimensions were measured with an ocular micrometer for 10 eggs from one to four egg masses produced by 27 females (1992). Eggs were measured as soon as possible after spawning and before the first egg/ mass began to show the elongation associated with the onset of cleavage (within 24 h of spawning; Gibson & Chia 1989). Data were analyzed with a one way Analysis of Variance (ANOVA) to reveal differences in egg size among females. Linear regression was used to test for a relationship between egg size/ female and characteristics of offspring, such as the mean percent of juvenile hatchlings or the mean time of metamorphosis for hatched veligers produced by each female. As absolute differences in egg size were small within an egg mass, the fate of individual eggs was not examined.

Statistical analysis

Descriptive statistics on individual traits were performed with Statview™ SE + Graphics (Abacus Concepts, 1988) on Macintosh. Genetic data were analyzed with SPSS-

X Release 3.0 with a modified nested ANOVA (UANOVA procedure) with males nested within years and females nested within males and years. Males and females were designated as random (Lawrence 1984). The results from this UANOVA were used in the statistical model

$$X_{ijk} = \mu+S_i+D_{ij}+E_{ijk}$$

where X_{ijk}= the record of the k^{th} offspring of the j^{th} female mated to the i^{th} male, μ=common mean, S_i=effect of i^{th} male, D_{ij}=effect of the j^{th} female mated to the i^{th} male, and E_{ijk}=uncontrolled environmental and genetic deviations attributable to offspring. This model assumes that individuals are chosen at random from a reference population that is a non-inbred random mating population (Falconer 1960). As female size may affect certain traits, weight at the beginning of the spawning season was incorporated in the model.

Nested ANOVA partitioned the phenotypic variance into components determined by sire components (σ^2_s), dam components (σ^2_d) and progeny components (σ^2_w). The composition of the mean squares is partitioned as:

Source of Variation	df	MS	Composition of MS
between sires	s-1	MS_S	$=\sigma^2_w+k\sigma^2_d+dk\sigma^2_s$
between dams within sires	s(d-1)	MS_d	$=\sigma^2_w+k\sigma^2_d$
between progeny within dams	sd(k-1)	MS_w	$=\sigma^2_w$

where s=number of sires, d=number of dams per sire, and k=number of progeny per dam (= number of egg masses). Phenotypic variance is the sum of the variance attributable to each of the three components ($V_p = \sigma^2_t = \sigma^2_s+\sigma^2_d+\sigma^2_w$).

The genotypic expression of a trait is a composite of differences in the average effects of underlying alleles (V_A, additive genetic variance), allelic interactions, combinations and dominance (V_{NA}, non-additive genetic variance), as well as environmental components such as sampling error, nutritional differences, seasonal differences, etc. Half-sib variance components (σ^2_s) include 1/4 V_A. Full-sib variance components (σ^2_d) include 1/4 V_A + 1/4 V_{NA} and thus are inflated. The within progeny variance component (σ^2_w) is equal to the total variance minus the covariance among progeny, and includes 1/2 V_A + 3/4 V_{NA} and all of the environmental effects. Additive genetic variance (V_A) was estimated as four times the covariance attributable to half sibs (= $4\sigma^2_s$). Non-additive genetic variance (V_{NA}) was estimated as the covariance of full sibs minus twice the covariance of half sibs (= $((\sigma^2_s + \sigma^2_d) - 2(\sigma^2_s))$; Rawson & Hilbish 1990).

Heritability was estimated as

$$h^2 = 4(\sigma^2_s)/ \sigma^2_t$$

or the additive genetic variance attributable to the sires over the total variance observed (Becker 1975). This provided an estimate of narrow-sense heritability and expresses the proportion of phenotypic variation (similarity between parent and offspring) upon which directional selection can act. Heritabilities estimated from the dam components are inflated by non-additive variance and thus represent broad-sense heritabilities (the overall variance in the trait attributable to all genetic effects). Estimates of h^2 were set to 0 if the variance component was negative. Standard errors were calculated as by Becker (1975) for a hierarchical design as S.E.$(h^2) = \sqrt{\text{var}}$.

C. Parental environment

Adult *H. callidegenita* were reared on one of two feeding regimes: group one (full rations) had food available continuously throughout a 40 d period, and group 2 (half rations) had food provided for 24 h alternating with food deprivation for 24 h throughout the experimental period. Food supplied was *Ulva* sp. attached to rocks collected from the mid-intertidal zone at Friday Harbor Laboratory. Although *H. callidegenita* ingest *Ulva* fragments, this source primarily provided a substrate for grazing both diatoms and bacterial film. When food was removed, *Ulva* and rocks were taken out of the cages and the cages themselves scrubbed with freshwater to remove accumulated diatoms and bacteria. Cages were then re-submerged in flowing, un-filtered seawater that did allow water-borne diatoms and bacteria to enter the cage. A deprivation interval of 24 h was considered adequate because slugs left without food for 3-4 d became completely inactive. Therefore, even though food was not completely excluded during this experiment, animals were considered to be energetically stressed.

Animals were collected from Padilla Bay immediately prior to the onset of the experiment (July 1990). Eight animals were maintained with full-food rations and 18 with half-rations. Every egg mass produced during the 40 d experimental period was collected and cultured as above. Number of hatchlings, length of the hatching period, and the percentage of juvenile hatchlings were determined.

D. Comparison among populations

Egg masses were collected from three sites in Washington State in June 1992: Padilla Bay, Chuckanut Bay, and Spencer Spit (Figure 5.1). Chuckanut Bay is at the mouth of the same estuarine system as Padilla Bay and is located approximately 21 km from the Padilla Bay site. It is similar to Padilla Bay in that it is composed of a large

mudflat dominated by *Zostera marina* and *Z. japonica* . Spencer Spit is the more distant site (approximately 32 km from Padilla Bay). It is a shallow, isolated lagoon with restricted access to open water, and never exposed at low tide. Dominant flora are *Ulva* sp. and mats of the filamentous green alga *Chaetamorpha linum*. As egg mass size is known to affect other traits of the mass (Chapter 2), I attempted to select masses that were approximately the same size, although was restricted at the Spencer Spit site to large masses as smaller masses were not available. In the laboratory, 20 egg masses/ site were chosen based on developmental stage (those selected had not yet undergone the second cleavage) in attempts to use masses synchronized in terms of time of spawning. Egg masses were cultured in 50 ml of 1 µm filtered seawater at 17 C. Number of hatchlings, length of the hatching period, and the percent of juvenile hatchlings determined for each egg mass as described above.

RESULTS

A. Life history traits

Description

Individual traits and differences between years

Life history traits were measured in two groups of animals, one group maintained in 1991 (36 females produced a total of 232 egg masses) and one in 1992 (33 females produced a total of 236 egg masses). Peak spawning occurred from August to October in 1991 and from May to August in 1992. During the course of this experiment, females produced a mean of 6.98 ± 2.45 egg masses, ranging from 1 to 14 masses/ female. The number of masses/ female was not significantly different between the two years (unpaired two-tail t-test, df=65, t=-1.2496). Four of the five life history traits measured showed significant differences between the two years (Table 5.2), although the actual differences were not large. Length of the embryonic period was the most conservative trait and showed least variation overall. In 1992, females spawned earlier in the summer, had a shorter spawning interval and produced smaller egg masses that hatched over a shorter period (Table 5.2). The percentage of hatchlings released as juveniles was 85.76% and 90.39% for 1991 and 1992 respectively, ranging from 33.91 to 100% overall (Table 5.2). The mean (±S.E.) percent juveniles produced by individual females ranged continuously from 62.72 ± 1.14 to 99.70 ± 0.06 % juveniles over both years.

Influence of female size on individual traits

Females in the 1991 season were 370 ± 11 mg (X ± S.E., range 210 to 520 mg) at the onset of their reproductive period and those of the 1992 season were 361 ± 15 mg

(range 212 to 767 mg, or 13 to 26 mm in length). The effects of female size on traits of the first EM produced were examined for both years with a linear regression. Only the first egg mass was considered so differences among females in growth rate throughout the reproductive season would not confound the results. Data were considered separately for each year if significant year differences were found (Table 5.2). Spawning interval, length of the embryonic period and the percent of juvenile hatchlings were not affected by female size (Table 5.3). Both the duration of the hatching period and number hatched/ EM increased with female size in 1991 but not in 1992 (Table 5.3; Figure 5.3). In 1991, female size accounted for most of the variance observed in number hatched and approximately half for duration of the hatching period (Figure 5.3).

Seasonal variation

I examined variation in the expression of each trait throughout an individuals entire spawning season by comparing traits of sequentially produced egg masses for each female with a linear regression. This analysis provides a broad estimate of change throughout the entire spawning season. Most egg masses were considered but a few masses were excluded because of a high incidence of embryo mortality (> 2%). A maximum of 10 egg masses were considered/ female.

The interval between spawning events gradually increased throughout the spawning period in 1991 although the overall variance was much greater than that accounted for by this model (r^2=0.04; Table 5.4); no significant differences were found in 1992. Embryonic period also increased slightly throughout this period. Hatching period increased slightly in 1992 but not significantly in 1991. Number hatched increased in both years with sequential spawning events, but again, only a small portion of the overall variance is accounted for by the model (r^2=0.06 in 1991 and 0.11 in 1992; Table 5.4). The percent of juveniles increased throughout the spawning period in 1992 (r^2=0.02; Table 5.4); no significant changes were noted in 1991.

Egg size

Egg size was determined for 27 females in 1992. Mean egg diameter was 262 ± 15 µm (n = 466 eggs). Egg size was significantly different among females (Table 5.5). Females producing larger eggs did not have a higher percent of juveniles at hatching (Table 5.5).

Genetic determination of life history traits and estimates of heritability

Genetic analysis indicated that the variance for some of the traits examined showed significant differences among sires (embryonic period and the percent of juvenile hatchlings) or dams (spawning interval, hatching period, number hatched, and the percent of juvenile hatchlings; Table 5.6). Variance components associated with each trait indicate if the major genetic effect is additive genetic variance, in which case σ^2_s and σ^2_d will be similar (e.g., the percent of juvenile hatchlings), or if non-additive effects are also playing a role in which case the dam component will be inflated (e.g., spawn interval, hatching period, number hatched; Table 5.6).

Variance components were decomposed into additive (V_A) and non-additive variance (V_{NA}) and indicated a strong non-additive contribution to spawning interval and a lesser contribution to length of the hatching period and number of hatchlings/ egg mass (Table 5.6). This pattern is as expected given the likelihood of a strong maternal effect on spawning frequency and size of the egg masses produced. Interestingly, metamorphosis within the egg mass (or, the percent of juvenile hatchlings) showed additive genetic variance only, with apparent equal contributions from sire and dam.

Number hatched was heritable through sires. Spawning interval, hatching period and number hatched were heritable through dams (broad sense heritability). Dam estimates were inflated through non-additive variance. The percent of offspring hatching as juveniles was highly heritable through both sires and dams. High standard errors associated with these heritability estimates fall within the range of values expected for these kinds of estimates (Becker 1975). Greater standard errors associated with sire estimates reflect the fact that this experimental design contained fewer sires than dams.

B. Time of metamorphosis

Description of individual traits and effects of female size

Two additional estimates of metamorphosis were considered: time of metamorphic competence after hatching, indicated by metamorphosis of larvae in the presence of a suitable inducing compound (egg mass jelly), and the ability of hatched larvae to delay metamorphosis if an inducer is withheld. Time of metamorphosis post-hatching ranged from 1 to 30 d among all clutches (Table 5.7A). The mean response time for the first veliger/ clutch to metamorphose in the presence of a metamorphic inducer was 3.66 d and metamorphosis was completed by 13.65 d for all siblings/ clutch (Table 5.7A). Metamorphosis could be delayed 12 d (to a maximum of 42 d post-hatching) in the absence of an inducing compound. Metamorphosis was significantly delayed throughout the metamorphic period if inducer was withheld (Table 5.7B); the first metamorphosis / clutch

occurred 4.78 d after hatching, the mean time to completion of metamorphosis was 20.63 d (Table 5.7A).

Offspring of larger females had an increased ability to delay metamorphosis early in the planktonic period (Table 5.3). Time of metamorphosis in the presence of a metamorphic inducer was not affected by female weight (Table 5.3).

Spawn time (examined through analysis of sequentially produced egg masses/ female) did not significantly effect time of metamorphosis of hatched larvae or on their ability to delay metamorphosis (Table 5.4 B, C). Mean egg size / female had no effect on mean time of metamorphosis or ability to delay metamorphosis of offspring (Table 5.5B).

Genetic basis for time of metamorphosis and estimates of heritability

Only three of the traits examined showed significant differences among either sires or dams (Table 5.8). Time of metamorphosis of the first veliger/ clutch showed significant differences among sires with estimates of heritability through sire effects of 0.35 (Table 5.8), primarily derived from additive genetic variance. Completion of metamorphosis by all veligers/ clutch showed significant differences among dams, with a broad sense heritability of 0.76. Non-additive genetic variance had approximately 2.5 times the contribution of additive genetic variance in determining the expression of this trait.

The ability to delay metamorphosis showed significant differences only in dam components for half of the veligers/ clutch ($h^2 = 0.66$) with non-additive genetic variance having the major role.

It is possible that additional traits are also heritable but this was not evident because so few sires were used in this study (1992 only, n=11 males). However, this experiment indicated that the onset of competence after hatching took place over a relatively long period of time within any one clutch (up to 30 d), with additive genetic variance affecting the onset of competence early in the planktonic period and non-additive genetic variance influencing the time of competence later in the planktonic period. The ability to delay metamorphosis was primarily determined through non-additive genetic variance.

C. Effects of parental environment on the percent of juvenile hatchlings

Parental environment was manipulated by culturing animals on two feeding regimes. Ten *H. callidegenita* were maintained in the continuous presence of food and produced a total of 27 egg masses, with a mean response of 2.80 ± 1.18 egg masses/ female (range 0 - 5 egg masses) over a period of 40 d. Eighteen animals maintained on half rations of food produced a total of 46 egg masses with a mean of 2.55 ± 0.78 egg masses/ female (range 1 - 4). The percent of juvenile hatchlings differed significantly between these

two groups such that animals on half-rations produced fewer juveniles while number of hatchlings and the length of the hatching period showed no differences (Table 5.9). *H. callidegenita* on full food rations showed no change in the percent of juvenile hatchlings throughout the experimental period (Figure 5.4A). *H. callidegenita* fed half rations showed an increase in the percent of juveniles produced from an initial value which was slightly lower than full-ration animals (Figure 5.4B). There were significant differences between groups in the percent of juveniles released from egg masses spawned in the first 10 d (unpaired two-tail t-test, df=26, t=-2.25, $p<0.05$) but not in the last 10 d of the experimental period (df=15, t=0.16).

D. Inter-population differences in percent of juvenile hatchlings

Egg masses were collected from seagrass beds at three sites: Padilla Bay, Chuckanut Bay and Spencer Spit (Figure 5.1). Egg masses collected at Padilla Bay showed the highest incidence of juvenile hatchlings and those from Spencer Spit the lowest (Figure 5.5). A one way ANOVA indicated significant differences among the three field sites overall (Table 5.10A). The Scheffé procedure for unplanned comparisons of means (Day & Quinn 1989) revealed significant differences in the percent of juvenile hatchlings between Padilla Bay and Spencer Spit, geographically the most widely separated sites. Egg masses from the Chuckanut Bay population showed proportions of juvenile hatchlings intermediate between these two populations, but not significantly different from either (Table 5.10B).

Egg masses collected from Padilla Bay were also compared with those spawned at the same time in a laboratory population of animals originally collected from Padilla Bay, and used in the genetic crosses discussed above. These animals had been collected 9 months previously and were maintained under conditions that were considerably removed from those of the natural population. An unpaired, two-tailed t-test did not reveal significant differences between these two groups (Figure 5.5).

DISCUSSION

Life history theory in benthic marine invertebrates has relied on observation of the phenotypic expression of traits and analysis of variation in these traits either within or among species. The phenotypic expression of a trait is determined genetically and is additionally modified by environmental effects and correlations with both morphological and other life history traits. Heritability describes the extent to which a character is transmitted from parent to offspring and therefore provides an estimate of potential

responses of a population in the expression of that character as well as potential responses to selection (Nyquist 1991). Life history traits generally have been thought to have low heritabilities because natural selection would erode genetic variation in traits closely related to fitness (Fisher 1958), or alternatively, because the expression of life history traits relies on the expression of underlying metric traits (i.e., the effects of female size on fecundity) and therefore is subject to the environmental effects on both (Price & Schluter 1991). However, recent heritability studies have estimated values that are much higher than had been anticipated indicating that life history traits may be based on sufficient genotypic variation for rapid action of natural selection (Roff 1992).

Reproductive traits

Life history traits were described for the two laboratory populations of the opisthobranch *Haminaea callidegenita* (maintained in 1991 and 1992). Females were approximately the same size at the onset of their spawning period in both years, although spawning occurred later in the summer in 1991 than in 1992. This probably reflects differences in collection time the previous fall as well as differences in growth throughout the winter, causing 1991 females to attain size of maturity later in the year. Traits showed small absolute differences between the two years. Females maintained in 1992 spawned more frequently and produced smaller egg masses that took less time to hatch. The effect of female weight on the expression of various traits was different between the two years (i.e., hatching period, clutch size). These differences may reflect seasonal as well as individual differences as 1991 females spawned in to the fall, while in 1992 spawning was completed earlier in the year. These differences emphasize the importance of multiple year comparisons in the analysis of life history traits.

Embryonic period was the most conservative trait and showed little variation overall, no differences between the two years and was not influenced by female size. This suggests that duration of the embryonic period is possibly subject to internal constraints through ontogeny or physiology, and may provide little scope for the action of selection. The duration of the embryonic period did increase slightly as the spawning season of each female progressed, although the correlation coefficient for this regression was very small (r^2=0.07; calculated for both years). The source for this increase is not clear but may suggest a changing reproductive condition of females throughout the spawning season. It is unlikely to reflect changes in ambient temperature throughout the summer as egg masses were cultured under constant conditions. Length of the embryonic period showed a very low level of heritability (0.08 ± 0.06) indicating that it would respond very slowly to selection, and supporting the suggestion that it is a highly conservative trait in this species.

Length of the hatching period was variable between years, among females, and seasonally. As hatching period increases with increases in egg mass size (Chapter 2) the increase in hatching period throughout the spawning season of an individual probably reflects female growth during this period. Hatching period was heritable through sires and was strongly influenced by non-additive genetic effects. This may provide flexibility to duration of the benthic, encapsulated period that has been considered as a period of relative "safety" to the developing larvae (Pechenik 1979; see also Rawlings 1990).

Clutch size (#hatchlings/ egg mass) changed as did hatching period with both year and seasonal effects, as might be predicted given the high degree of association between the two traits. Clutch size was also influenced by female size, as expected because the ability of larger females to produce larger egg masses has been well established in opisthobranchs (Todd 1979; Todd & Havenhand 1988). Clutch size was also highly heritable and showed strong influences of non-additive genetic effects. Fecundity is known to be highly heritable in the polychaete *Streblospio* (Levin et al. 1991; the only such study in benthic marine invertebrates) as well as in some insects, such as the flour beetle *Tribolium* (Riddle et al. 1986) and the milk weed bug *Oncopeltus* although in this species it is only detectable under certain environmental conditions (Groeters & Dingle 1987).

The interval between spawning events showed a slight seasonal increase (1991 only) and was not influenced by female size. Heritability through sires was not detected, possibly because of the overwhelming variance caused by non-additive genetic effects, detected through dams. Interclutch interval is known to be heritable at very low levels in some insects (e.g.,*Oncopeltus*; Hegman & Dingle 1982).

Time of intracapsular metamorphosis

Time of metamorphosis within the egg mass was strongly heritable with almost no effects from non-additive genetic variance. This may suggest that this trait has a relatively simple genetic basis, involving few genes (F. Yeh, pers. comm.). The immediate result of intracapsular metamorphosis is to determine the number of larvae that metamorphose within the egg mass, but it also influences the genetics of the entire population by determining the percent of offspring with a potential for dispersal from or recruitment to the parental population. These data predict genetic differences are likely among populations based on limited exchange of dispersive propagules (i.e., swimming veligers) (Burton 1983, 1986), and are supported by inter-population differences in the percent of juvenile hatchlings.

The mean percent juveniles hatching/ egg mass was different for each of the three collection sites but only two sites were significantly different (Padilla Bay, Spencer Spit). This suggests a low level of exchange of propagules between Padilla Bay and Chuckanut

Bay, which is likely given the close proximity of the two sites (Figure 5.1). The similarity between Spencer Spit and Chuckanut Bay may also indicate limited gene flow between these sites, although a reason for lack of similar exchange with Padilla Bay is not clear. A more likely explanation is suggested by the topography Spencer Spit, a shallow lagoon appearing to have restricted seawater exchange with open water (connected by a single, shallow channel less than 1 m wide). The low level of intracapsular metamorphosis occurring in the Spencer Spit population could be a result of founder effects, whereby the original colonizers were planktonic larvae, and possibly were genetically "late" to metamorphose. Gene frequencies producing low levels of intracapsular metamorphosis may have remained in the Spencer Spit population if 1) the population was established relatively recently, or 2) planktonic larvae produced in the population recruit directly into the parental population, a possibility as veligers may be retained by the dense mats of *Chaetamorpha* and *Ulva* that fill most of the lagoon.

Time of intracapsular metamorphosis was not altered by parental environment in the long term, as indicated by similarities between the field *vs.* laboratory populations of Padilla Bay *Haminaea*, in which the laboratory population had been maintained under extremely artificial conditions for at least 8 months prior to data collection. This suggests that parental genetics play a much greater role in determining the percent of juvenile offspring than does parental environment.

Time of metamorphosis appeared to change over the short term if parents were maintained under stressful conditions involving unpredictable food availability. Food availability may not be an ecologically valid manipulation as it is improbable that food would be limited in nature (*H. callidegenita* eat diatoms and detritus, two sources which are abundant year round in seagrass beds, although doubtless change in composition). However, food was easily manipulated in the laboratory in these preliminary experiments. Stressed females produced fewer juveniles initially, then the percent of juveniles gradually increased to "control" conditions (Figure 5.6). In nature, this would result in females located in unpredictable or "poor" environments producing more dispersive propagules, and therefore broadcasting offspring to new and possibly better habitats. The subsequent recovery to a perhaps genetically pre-determined level is supported by the lack of environmental effect after maintenance of animals in the laboratory over a long period of time.

Estimates of intracapsular metamorphosis may be confounded by the possibility of habituation of the veligers to metamorphic inducer because inducer (egg mass jelly) was continuously present in the culture chambers. This phenomenon has been observed in two other molluses, *Phestilla sibogae* (Hadfield 1980) and *Haliotis rufescens* (Tradipo-

Rosenthal & Morse 1986) but not in *Crepidula fornicata* (Pechenik & Gee 1993). Habituation seems unlikely to affect metamorphosis in *Haminaea callidegenita* because egg mass jelly is present throughout the entire pre-hatching larval period yet many sibs/ egg mass will respond before hatching; also, it is not necessary to desensitize larvae before they will respond.

Metamorphosis after hatching

After hatching, time of metamorphosis determines the length of the planktonic period and consequently the potential distance an offspring can disperse from the parent. Time of metamorphosis after hatching was heritable through sire effects throughout the planktonic period, and through dam effects near the end of the planktonic period, suggesting the importance of maternal effects late in the planktonic period. Metamorphosis after hatching was independent of female size, egg size, and seasonal change.

The ability of larvae to delay metamorphosis was heritable through the dam component and was detectable only during the mid-planktonic period, again with the primary basis being non-additive effects. It is likely that additional traits are heritable but were undetected with the small number of sires used in this study. Larval ability to delay was independent of egg size and seasonal change and was slightly affected by female size early in the planktonic period. The potential for larger females to produce offspring with a slighter greater ability to delay metamorphosis suggests energetic state of spawning females may be involved to determining ability to delay metamorphosis. Ability to delay may rely on energetic differences among eggs, and hence ultimately on nutritional state of the spawning female. I was unable to detect this through a preliminary comparison of egg size, although egg size does not necessarily correlate with organic content in other taxa (McEdward & Coulter 1987).

Variation in time of metamorphosis within one culture has been noted for many species of benthic marine invertebrates, but the species examined have primarily been planktotrophic (i.e., producing feeding larvae), so variance is easily attributed to differences in feeding and growth. There have been a few attempts to detect heritability for metamorphosis. In the lecithotrophic opisthobranch *Phestilla*, no genetic component to time of competence was detected even after raising this species through 21 generations (Hadfield, 1984). In cyprid larvae of the barnacle *Balanus amphitrite*, settlement and metamorphosis were determined by non-additive genetic effects; additive genetic effects, and therefore heritability, could not be detected (Holm 1990). Studies on polychaets have determined a heritable component for duration of the planktonic period (*Streblospio benedicti* ; Levin et al.. 1991) and for preference of substrates inducing settlement in the

polychaete *Spirorbis borealis* (including additive and non-additive effects; Mackay & Doyle 1978). Duration of the free-swimming larval phase has also been found to be heritable in some vertebrates, such as the anuran *Hyla gratiosa* (Travis 1980).

Time of metamorphosis is heritable in *Haminaea callidegenita* with strongest effects early in the metamorphic period (i.e., within the egg mass). Non-additive genetic effects have the greatest effect late in the metamorphic period (to 14 d after hatching). The threshold defining time of metamorphosis appears to be complex in origin. A possible mechanism determining length of the planktonic period is the imposition of energetic restrictions on the underlying genetic sources of variance described above, although this remains to be tested. These veligers are non-feeding and are probably constrained by energy resources defining the period through which they are able to delay feeding and still retain sufficient energy reserves to complete metamorphosis.

Conclusion

Several life history traits in *Haminaea callidegenita* are heritable and are variously influenced by environmental factors (female size, spawn season, parental environment). There were year differences in both the phenotypic expression of these traits as well as in the effects of female size, emphasizing the importance of analysis of traits over more than one year. Some traits are highly conservative (e.g., embryonic period) while others are variously determined by additive and non-additive effects and influenced by environment (e.g., spawn interval). Time of metamorphosis is highly heritable, especially early in the larval period, and therefore may respond rapidly to selection. Late metamorphosis and delayed metamorphosis is influenced by non-additive genetic effects and may be determined by energetic constraints.

Table 5.1: Definition of *Haminaea callidegenita* life history traits. EM = egg mass.

TRAIT	DEFINITON
Reproductive traits and intracapsular metamorphosis (1991 and 1992):	
Spawn interval	- number of days between spawning events/ female
Number hatched	- number of hatchlings (both veligers and juveniles) / EM
Embryonic period	- number of days from oviposition to first day of hatching/ EM
Hatching period	- number of days from the onset to completion of hatching/ EM
Percent juveniles	- the percent of offspring / EM that metamorphosed within the egg mass and hatched as juveniles (arcsin transformed)
Time of metamorphosis of hatched veligers (metamorphic inducer present; 1992 only):	
Onset of competence	- number of days between hatching and the first metamorphosis of a hatched veliger/ EM (= minimum planktonic period)
Median time of metamorphosis	- number of days between hatching and metamorphosis of half the hatched veligers/ EM
Maximum time of metamorphosis	- number of days from hatching until metamorphosis was completed by all veligers/ EM
Time metamorphosis could be delayed by hatched veligers (inducer absent; 1992 only):	
Minimum delay period	- number of days from hatching to the first metamorphosis of a hatched veliger/ EM in the absence of inducer
Median delay period	- number of days from hatching until half the hatched veligers/ EM had metamorphosed
Maximum delay period	- number of days from hatching to completion of all veligers/ EM cultured in absence of inducer (= maximum planktonic period)

Table 5.2: Summary of life history traits for female *Haminaea callidegenita* for 1991 (top value; n=232 egg masses) and 1992 (bottom value; n=236 egg masses). Unpaired t-values are listed for year comparisons. EM= egg mass. ***p<0.001

TRAIT	YEAR	X±S.E.	MIN	MAX	YEAR COMPARISON dF	T VALUE	p
Spawning interval (d)	1991	8.22±0.39	0	38	466	4.89	***
	1992	5.74±0.32					
Embryonic period (d)	1991	14.25±0.09	9	19	466	-1.39	ns
	1992	14.43±0.08					
Hatching period (d)	1991	6.04±0.15	1	13	466	12.49	***
	1992	3.81±0.08					
Number of hatchlings/ EM	1991	373.92±9.83	32	825	466	4.48	***
	1992	317.36±7.95					
Percentage juveniles hatching/ EM	1991	85.67±0.88	33.91	100	466	-4.63	***
	1992	91.79±0.65					

Table 5.3: Influence of female size on: A) reproductive traits in *Haminaea callidegenita* (68 females, 1991 and 1992); B) time of metamorphic competence (33 females, 1992 only); and C) ability to delay metamorphosis (33 females, 1992 only) in hatched veligers. Results are from a linear regression of effects female size on each trait. Data for each year are considered separately if a significant difference among years in expression of the trait was found (see Table 5.2). Size is female wet weight (mg) at the onset of the reproductive period. Traits are defined Table 5.1. Only the first egg mass (EM) spawned by each female was considered in the analysis. *** $p<0.001$, ** $p<0.01$

	YEAR	df	r^2	F	p	
A. Reproductive traits						
Spawn interval (d)	1991	1, 34	0.02	0.66	ns	y= 0.01x+ 5.13
	1992	1, 32	0.00	0.01	ns	y= 0.00x+ 4.16
Embryonic period (d)	BOTH	1, 66	0.01	0.92	ns	y= 0.00x+13.77
Hatching period (d)	1991	1, 34	0.47	29.77	***	y= 0.02x- 1.05
	1992	1, 32	0.05	1.59	ns	y= -0.00x+ 2.95
Number hatched/ EM	1991	1, 34	0.88	225.17	***	y= 1.68x-249.55
	1992	1, 32	0.04	1.13	ns	y= 0.17x+221.82
Percent juveniles	1991	1, 34	0.01	0.54	ns	y= 0.02x+ 55.34
	1992	1, 32	0.01	1.00	ns	y= -0.01x+ 77.49
B. Time of metamorphosis post-hatching (metamorphic inducer present)						
1st metamorphosis/ clutch		1, 32	0.04	1.22	ns	y= 0.00x+3.947
50% metamorphosed/ clutch		1, 32	0.004	0.14	ns	y= 0.00x+6.375
100% metamorphosed/ clutch		1, 32	0.05	1.75	ns	y= 0.01x+8.941
C. Ability to delay metamorphosis post-hatching (inducer absent)						
1st metamorphosis/ clutch		1, 31	0.15	5.45	**	y= 0.01x-0.623
50% metamorphosed/ clutch		1, 31	0.09	3.15	ns	y= 0.01x+4.427
100% metamorphosed/ clutch		1, 31	0.02	0.59	ns	y= -0.01x+24.85

Table 5.4: Effects of the order egg masses were spawned on: A) reproductive traits; B) time of metamorphic competence; and C) ability of larvae to delay metamorphosis in egg masses produced sequentially throughout the spawning period for individual females. Results are from a linear regression of effects of spawn sequence on each trait . Traits are defined in Table 5.1. A. Data for 1991 (top value) and 1992 (bottom value); data for each year are considered separately if significant differences between the years were noted (see Table 5.1). B, C: Data for 1992 only. ***p<0.001, ** p<0.01, *p<0.05

TRAIT	YEAR	df	r^2	F	p	
A. Reproductive traits						
Spawn interval (d)	1991	1, 243	0.04	11.14	***	y= 0.21x+7.51
	1992	1, 221	0.00	0.06	ns	y= 0.03x+5.47
Embryonic period (d)	BOTH	1, 465	0.07	33.82	***	y= 0.15x+13.71
Hatching period (d)	1991	1, 243	0.01	3.04	ns	y= 0.11x+5.54
	1992	1, 218	0.05	10.93	**	y= 0.12x+3.23
Number hatched/ EM	1991	1, 243	0.06	15.59	***	y=14.79x+314.84
	1992	1, 219	0.11	28.17	***	y=16.71x+239.17
Percent juveniles	1991	1, 243	0.00	1.09	ns	y= 0.17x+62.67
	1992	1, 216	0.02	4.42	*	y=0.40x+68.56
B. Time of metamorphosis (metamorphic inducer present)						
1st metamorphosis/ clutch		1, 133	0.01	0.91	ns	y= 0.08x+3.44
50% metamorphosed/ clutch		1, 133	0.00	0.41	ns	y= 0.08x+5.74
100% metamorphosed/ clutch		1, 133	0.00	0.46	ns	y= 0.26x+12.87
C. Ability to delay metamorphosis (inducer absent)						
1st metamorphosis/ clutch		1, 118	0.00	0.17	ns	y=–0.12x+5.08
50% metamorphosed/ clutch		1, 118	0.00	0.02	ns	y= 0.06x+9.09
100% metamorphosed/ clutch		1, 118	0.00	0.14	ns	y= 0.25x+20.15

Table 5.5: Size of eggs produced by *Haminaea callidegenita* females. A. Results of a one way ANOVA comparing egg size (as maximum egg dimension in µm) among females (n=27 females). B. Results of a linear regression of mean egg size/ female on three estimates of time of metamorphosis of her offspring. Estimates are the mean percent of juvenile hatchlings, mean time of metamorphosis (in days) of all hatched veligers/clutch in the presence of a metamorphic inducer (egg mass jelly), and ability of hatched veligers to delay metamorphosis if an inducer is withheld, also measured in days. *** $p<0.001$

A. Results of a 1 way ANOVA comparing egg size among females

n=466 eggs egg size X ± S.E. maximum diameter = 262.97±0.71 µm

SOURCE	df	SS	MS	F	p
between females	26	59309.53	2281.14	19.15	***
within females	439	52277.20	119.08		
total	465	111586.74			

B. Results of a linear regression of mean egg diameter by mean time of metamorphosis of offspring/ female

TRAIT	df	F	p	r^2	
Percent juveniles	1, 23	0.26	ns	0.01	y= 0.00x+49.41
Time of metamorphosis	1, 23	0.64	ns	0.03	y= 0.07x+1.2509
Ability to delay metamorphosis	1, 23	0.17	ns	0.01	y= 0.03x+5.1543

Table 5.6: Results from a nested ANOVA and summary of variance components (σ^2) for life history traits of *Haminaea callidegenita*. The relative contributions of additive genetic variance (V_A), non-additive genetic variance (V_{NA}) and heritabilities (h^2) are included. Data represent a hierarchical breeding design with females nested within males and males nested within years. *** $p<0.001$, ** $p<0.01$, * $p<0.1$. EM= egg mass.

TRAIT	SOURCE	DF	MS	F-RATIO	p	σ^2	V_A	V_{NA}	h^2±S.E.
Spawn interval (d)	Year	1	721.10	13.58	***				
	Sires	18	53.08	1.00	ns	-0.121	-0.484	8.25	0
	Dams	48	52.93	2.02	***	4.006			0.53±0.16
	Progeny	400	26.26			26.259			
Embryonic period (d)	Year	1	3.88	2.25	ns				
	Sires	18	2.25	1.40	*	0.040	0.16	-0.16	0.08±0.06
	Dams	48	1.40	20.6	ns	-0.099			0
	Progeny	400	2.06			2.056			
Hatching period (d)	Year	1	583.76	41.52	***				
	Sires	18	14.06	1.54	ns	0.182	3.89	6.63	0.19±0.21
	Dams	48	9.10	3.46	***	0.973			0.98±0.19
	Progeny	400	2.63			2.630			
Number of hatchlings/ EM	Year	1	374346.30	7.10	**				
	Sires	18	52739.82	1.22	ns	1542.30	6169.20	13930.28	0.34±0.06
	Dams	48	43351.43	3.07	***	5024.88			1.11±0.18
	Progeny	400	14114.11			11485.49			
Percent juveniles hatching/ EM	Year	1	1.04	2.31	ns				
	Sires	18	0.45	3.45	***	0.013	0.052	0.004	0.76±0.45
	Dams	48	0.13	3.34	***	0.014			0.85±0.25
	Progeny	400	0.04			0.039			

Table 5.7: Number of days between hatching and metamorphosis of *Haminaea callidegenita* veligers. A. Means (±S.E.) for each experimental group (1= metamorphic inducer is present, 2=metamorphic inducer is absent). B. Results of an unpaired two-tailed t-test between treatments (presence/ absence of metamorphic inducer). Time of metamorphosis is listed for three intervals of the planktonic period: i) the first metamorphosis/ clutch; ii) metamorphosis of 50% of the hatched veligers/ clutch; and iii) metamorphosis completed by all hatched veligers/ clutch. Egg masses were produced by females mated in a hierarchical design with females nested within males. *** $p<0.001$

A. Comparison of mean time of metamorphosis

GROUP	n	X±S.E.	MIN	MAX
1. Onset of metamorphic competence				
i. 1st metamorphosis	137	3.66±0.13	1	11
ii. 50% metamorphosed	137	5.95±0.19	2	13
iii. 100% metamorphosed	137	13.65±0.56	2	30
2. Metamorphosis delayed				
i. 1st metamorphosis	123	4.78±0.35	1	26
ii. 50% metamorphosed	123	9.22±0.48	2	26
iii. 100% metamorphosed	123	20.63±0.76	3	42

B. Comparison of time of metamorphosis in presence/ absence of inducer

	dF	T-VALUE	p
i. 1st metamorphosis	258	-3.20	***
ii. 50% metamorphosed	258	-6.51	***
iii. 100% metamorphosed	258	-7.56	***

Table 5.8: Results of a nested ANOVA and summary of variance components (σ^2) for time of metamorphosis after hatching in the presence of a metamorphic inducer (A) and the ability to delay metamorphosis in the absence of metamorphic inducer (B) in larval *Haminaea callidegenita* . Data are the number of days until metamorphosis occurred in 1) the first veliger/ clutchs, 2) 50% of the veligers/ clutch, and 3) 100% of the veligers/ clutch. The relative contributions of additive genetic variance (V_A), non-additive genetic variance (V_{NA}), and estimates of heritability (h^2) are also included. Data were obtained in a hierarchical breeding design with females nested within males, and males nested within years. ** $p<0.01$

TRAIT	SOURCE	DF	MS	F-RATIO	p	σ^2	V_A	V_{NA}	h^2±S.E.
A. Onset of metamorphic competence									
1. 1st metamorphosis	Sires	10	3.77	2.64	**	0.200	0.80	-0.20	0.35±0.21
	Dams	22	1.43	0.63	ns	-0.210			0
	Progeny	104	2.25			2.253			
2. 50% metamorphosed	Sires	10	6.26	1.49	ns	0.169	0.676	-0.84	0.15±0.19
	Dams	22	4.19	0.96	ns	-0.041			0
	Progeny	104	4.35			4.355			
3. 100% metamorphosed	Sires	10	98.9	1.56	ns	2.416	9.66	22.48	0.22±0.33
	Dams	22	63.43	1.99	**	8.036			0.76±0.31
	Progeny	104	31.9			31.899			
B. Metamorphosis delayed									
1. 1st metamorphosis	Sires	10	11.07	0.77	ns	-0.285	-1.14	0.08	0
	Dams	21	14.32	0.93	ns	-0.265			0
	Progeny	91	15.31			15.315			
2. 50% metamorphosed	Sires	10	49.00	1.15	ns	0.464	1.86	17.80	0.06±0.28
	Dams	21	42.70	1.76	**	4.915			0.66±0.37
	Progeny	91	24.22			24.223			
3. 100% metamorphosed	Sires	10	68.76	0.96	ns	-0.228	-0.91	1.54	0
	Dams	21	71.27	1.01	ns	0.156			0.01±0.29
	Progeny	91	70.69			70.686			

Table 5.9: Summary of the effects of parental environment on female reproductive characteristics. Females were maintained on full-food or on half-food rations (see text). Reproductive characteristics examined are percent of juveniles at hatching (as arcsin transformed percentages), number of hatchlings/ egg mass and hatching period (in days). Data were compared with an unpaired two-tailed t-test. **p<0.05

TRAIT		n	X±S.E.	df	t	p
Percent juveniles						
	Full ration	27	32.75±2.10	70	-2.40	**
	Half ration	45	26.44±0.03			
Number hatched/ EM						
	Full ration	27	360.33±35.67	70	-0.68	ns
	Half ration	45	332.57±49.56			
Hatching period (d)						
	Full ration	27	4.96±0.23	70	0.59	ns
	Half ration	45	5.24±0.32			

Table 5.10: Characteristics of egg masses collected from 3 field sites (P = Padilla Bay, C = Chuckanut Bay and S = Spencer Spit), as well as from a laboratory population originally collected from Padilla Bay. Traits considered are the percent of juvenile hatchlings/ egg mass (arcsin transformed percentages), number hatched/ egg mass, and the length of the hatching period/ egg mass in days. A. Comparison of each trait among the three field sites (one way ANOVA). B. Comparison among individual sites (Scheffé procedure for unplanned comparisons). C. Comparison of the Padilla Bay field and laboratory populations (unpaired two-tailed t-test). ***p<0.001, **p<0.05

A. 1 way ANOVA among 3 field sites

	SOURCE	dF	SS	MS	F-ratio	p
i. Percent juveniles						
	between sites	2	3749.41	1874.70	7.10	***
	within sites	52	13722.02	263.88		
	total	54	17471.43			
ii. Number hatched/ EM						
	between sites	2	603551.1	301775.5	10.8	***
	within sites	52	1450144.8	27887.4		
	total	54	2053695.9			
iii. Hatching period (d)						
	between sites	2	52	26	7.2	***
	within sites	52	187.9	3.6		
	total	54	239.9			

B. Comparison of traits among sites

TRAIT	SITE	F-test	p
i. Proportion of juvenile hatchlings	P x S	7.10	**
	P x C	2.29	ns
	S x C	1.54	ns
ii. Number hatched/ EM	P x S	3.40	**
	P x C	1.40	ns
	S x C	10.70	**
iii. Hatching period (d)	P x S	4.70	**
	P x C	0.005	ns
	S x C	5.80	**

C. Unpaired t-test between two Padilla Bay populations (field and laboratory)

TRAIT	dF	t	p
Proportion of juvenile hatchlings	33	-0.87	ns
Number hatched/ EM	33	-0.83	ns
Hatching period	33	1.99	ns

Figure 5.1. Collection sites for *Haminaea callidegenita* in Puget Sound, WA. Animals were maintained at Friday Harbor Laboratory, also in Puget Sound.

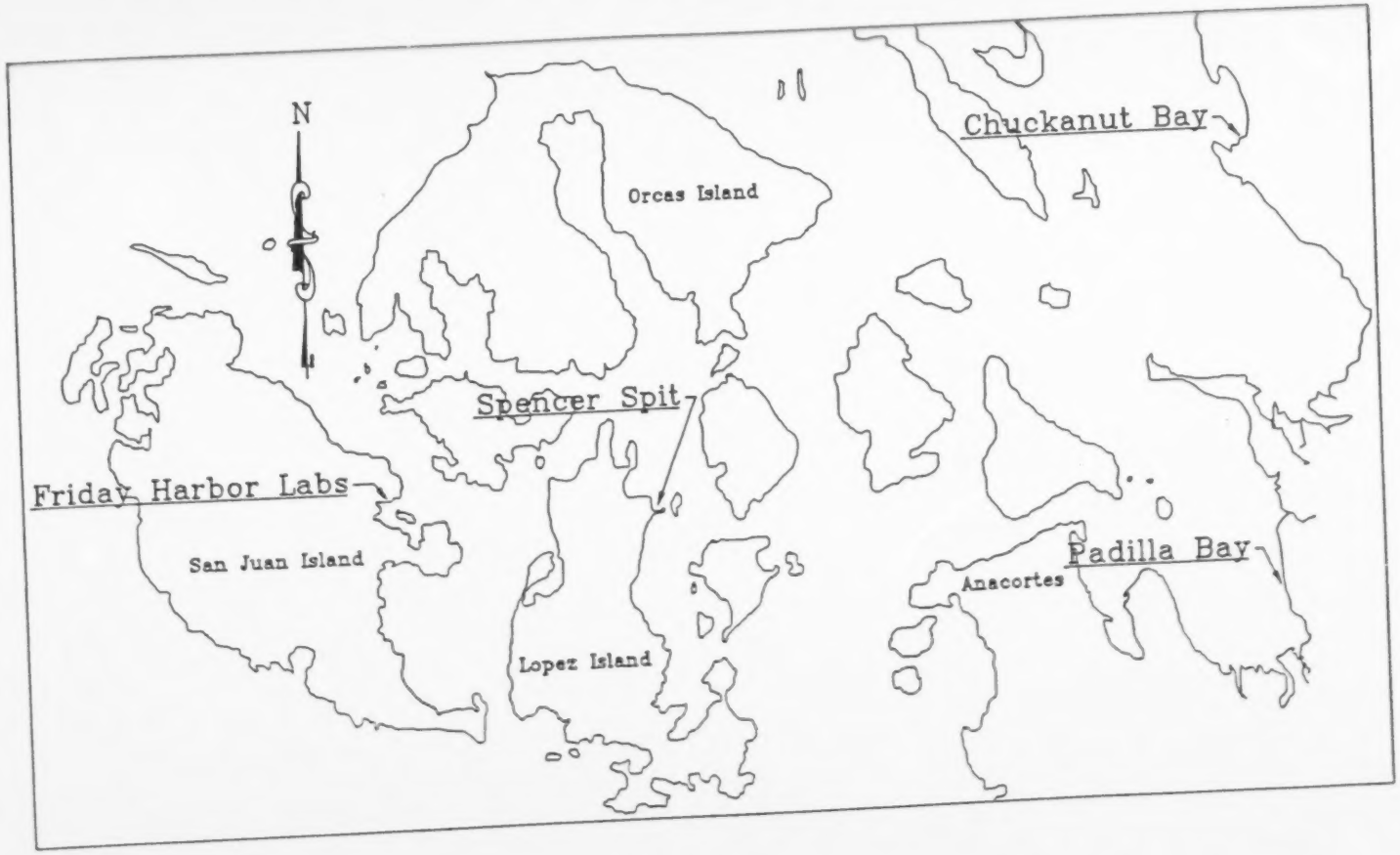
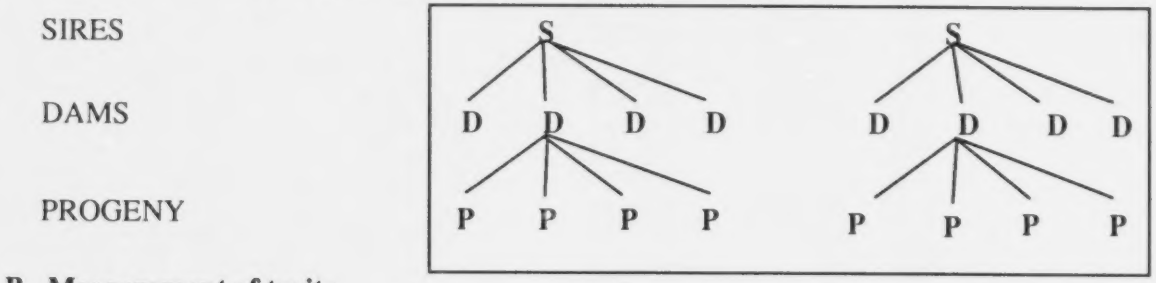


Figure 5.2: A. Breeding design for sib analysis (Lawrence 1984). B. Breeding design for sib analysis used in *Haminaea callidegenita*. Each male was mated with four females, and all progeny (= egg mass) were considered.

A. Hierarchical breeding design for sib analysis



B. Measurement of traits

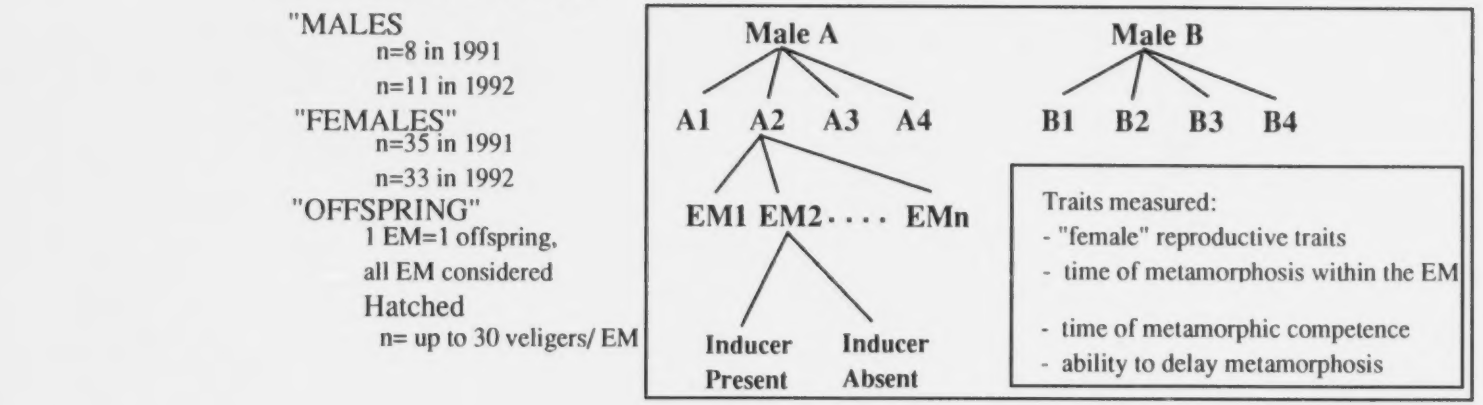
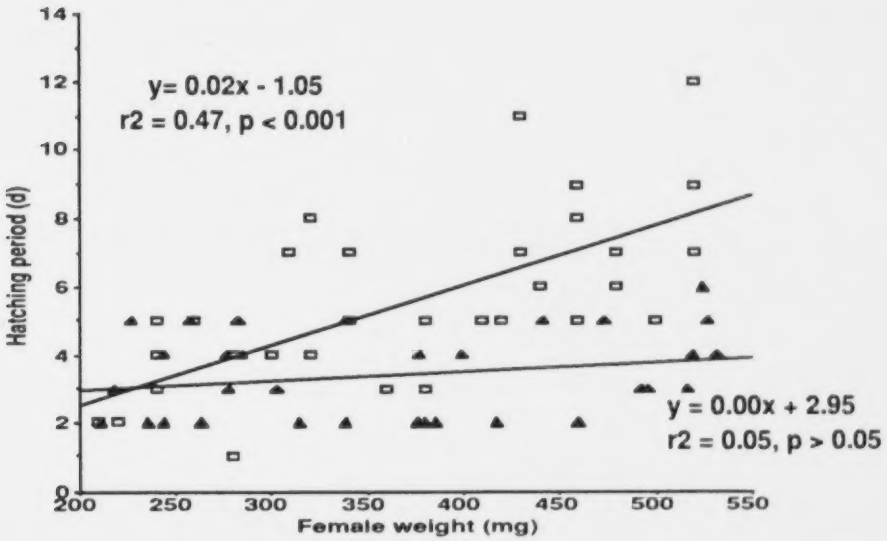


Figure 5.3. The effect of female wet weight (mg) on hatching period (A) and clutch size (B). Curves were estimated with linear regression for 1991 (open boxes) and 1992 (filled triangles) data.

A



B

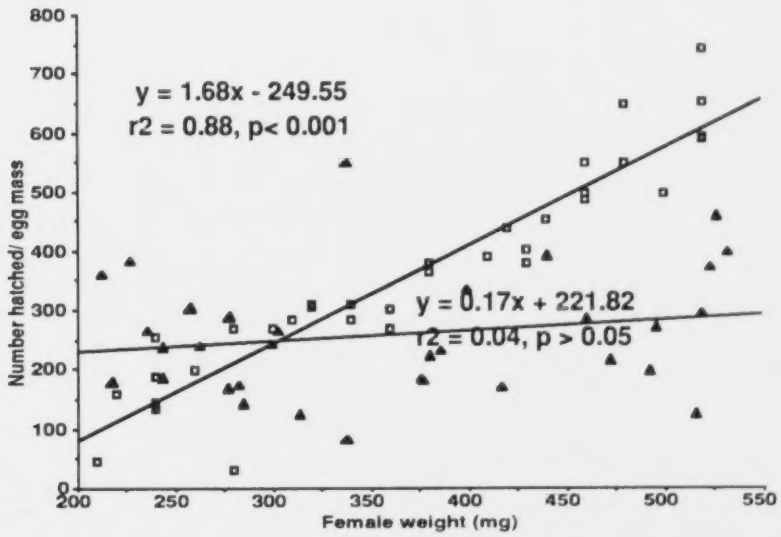


Figure 5.4. Effects of food availability on the percent juveniles hatching from egg mass produced by females on half food rations (A) and on continuous rations (B). Data are plotted by the day of the experimental period on which the mass was spawned. Curves were estimated with linear regression.

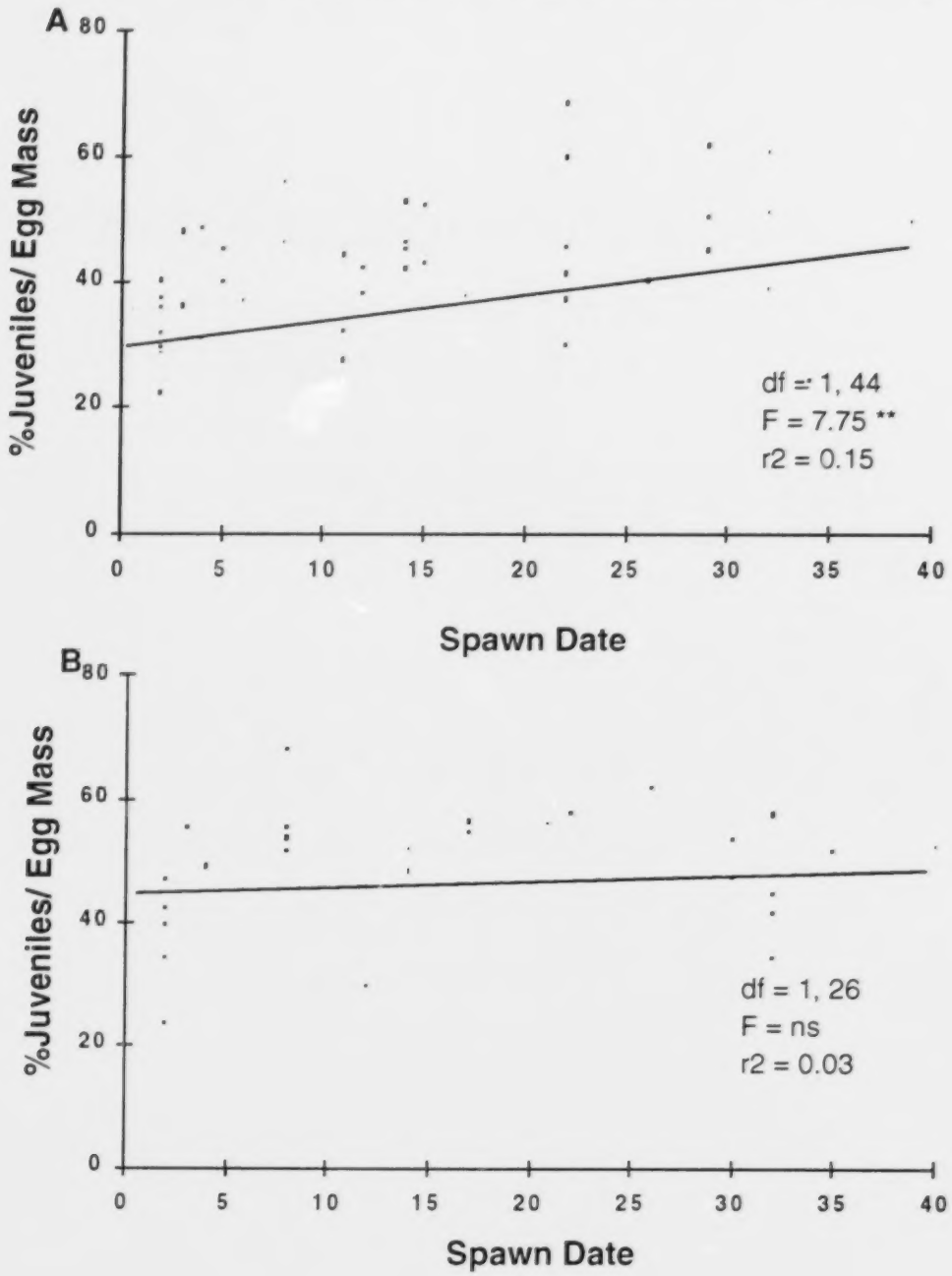
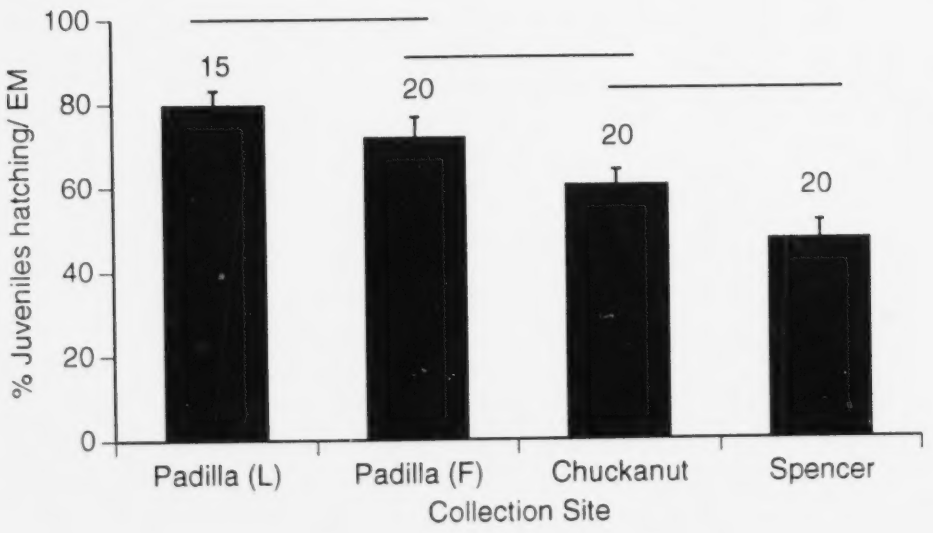


Figure 5.5. Proportion of juveniles hatching from egg masses collected from three field sites (Padilla Bay, Chuckanut Bay, Spencer Spit) and from a laboratory population originally collected from Padilla Bay). Values are X±S.E. for each site; sample size is above each column. Horizontal lines indicate no significant differences were found (see Table 5.10).



Literature Cited

Becker, W. A. 1975. *Manual of Quantitative Genetics*, 4th ed. Pullman, WA: Academic Enterprises. 190pp.

Burton, R. S. 1983. Protein polymorphisms and genetic differentiation of marine invertebrate populations. *Marine Biology Letters* **4**:193-206.

_____. 1986. Evolutionary consequences of restricted gene flow among natural populations of the copepod, *Tigriopus californicus*. *Bull. Mar. Sci.* **39**(2): 526-535.

Cassidy, P. M. and G. L. McKeen. 1986. Padilla Bay baseline water quality record. Report to NOAA/ OCRM/ MEMD by Western Washington University, Shannon Point Marine Center. 472 pp. Anacortes, Washington. Padilla Bay National Estuarine Research Reprint Series No. 2, 1990.

Caswell, H. 1983. Phenotypic plasticity in life history traits: demographic effects and evolutionary consequences. *Am. Zool.* **23**:35-46.

Day, R. and L. McEdward. 1984. Aspects of the physiology and ecology of pelagic larvae of marine benthic invertebrates. *In.* Marine Plankton Life Cycle Strategies. *Ed.*: K. A. Steidinger and L. M. Walker. Boca Raton, FL: CRC Press. pp. 93-120.

Day, R. W. and G. P. Quinn. 1989. Comparisons of treatments after an analysis of variance in ecology. *Ecological Monographs* **59**(4):433-463.

DeFreese, D. E. & K. B. Clark. 1983. Analysis of reproductive energetics of Florida Opisthobranchia (Mollusca: Gastropoda). *Int. J. Invert. Reprod.* **6**:1-10.

Falconer, D. S. 1960. Introduction to Quantitative Genetics. New York: Ronald Press Co. 365 pp.

Fisher, R. A. 1958. The Genetical Theory of Natural Selection 2nd ed. New York: Dover Publications, inc. 291 pp.

Gibson, G. D. & F.-S. Chia. 1989. Developmental variability (pelagic and benthic) in *Haminaea callidegenita* (Opisthobranchia: Cephalaspidea) is influences by egg mass jelly. *Biol. Bull.* **176**:103-110.

Groeters, F. R. & H. Dingle. 1987. Genetic and maternal influences on life history plasticity in response to photoperiod by milkweed bugs (*Oncopeltus fasciatus*). *Am. Nat.* **129**(3):332-346.

Hadfield, M. G. 1980. Habituation in metamorphic induction of larvae of *Phestilla sibogae* (Gastropoda). *Am. Zool.* **20**:955.

Hadfield, M. G. 1984. Settlement requirements of molluscan larvae: new data on chemical and genetic roles. *Aquaculture* **39**: 283-298.

Hegmann, J. P. & H. Dingle. 1982. Phenotypic and genetic covariance structure in milkweed bug life history traits. *In* Evolution and Genetics of Life Histories. *Ed.*: H. Dingle and J. P. Hegmann. Pp. 177-185. New York: Springer-Verlag.

Holm, E. 1990. Attachment behaviour in the barnacle *Balanus amphitrite amphitrite* (Darwin): genetic and environmental effects. *J. Exp. Mar. Biol. Ecol.* **135**:85-98.

Jablonski, D. J. & R. A. Lutz. 1983. Larval biology of benthic marine invertebrates: Paleobiological implications. *Biol. Rev.* **58**:21-89.

Lawrence, M. J. 1984. The genetical analysis of ecological traits. *In*: Evolutionary Ecology: the 23rd Symposium of the British Ecological Society Leeds 1982. *Ed.*: B. Shorrocks. Oxford: Blackwell Scientific Publications. pp 27-63.

Levin, L. A., J. Zhu, and E. Creed. 1991. The genetic basis of life history characters in a polychaete exhibiting planktotrophy and lecithotrophy. *Evolution* **45**(2):380-397.

Mackay, T. F. and R. W. Doyle. 1978. An ecological genetic analysis of the settling behaviour of a marine polychaete: 1. Probability of settlement and gregarious behaviour. *Heredity* **40**(1):1-12.

McEdward, L. R. & L. K. Coulter. 1987. Egg volume and energetic content are not correlated among sibling offspring of starfish: implications for life-history theory. *Evolution* **41**(4):914-917.

Mileikovsky, S. A. 1971. Types of development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. *Mar. Biol.* **10**:193-213.

Nyquist, W. E. 1991. Estimation of heritability and prediction of selection response in plant populations. *Critical Reviews in Plant Science* **10**(3):235-322.

Pechenik, J. A. 1979. Role of encapsulation in invertebrate life histories. *Am. Nat.* **114**:859-870.

Pechenik, J. A. and C. Gee. 1993. Onset of metamorphic competence in larvae of the gastropod *Crepidula fornicata* (L.), judged by a natural and an artificial cue. *J. Exp. Mar. Biol. Ecol.* **167**: 59-72.

Price, T. and D. Schluter. 1991. On the low heritabilities of life history traits. *Evolution* **45**(4):853-861.

Rawlings, T. 1990. Associations between egg capsule morphology and predation among populations of the marine gastropod *Nucella emarginata*. *Biol. Bull.* **179**: 312-325.

Rawson, P. D. & T. J. Hilbish. 1990. Heritability of juvenile growth for the hard clam *Mercenaria mercenaria*. *Mar. Biol.* **105**:429-436.

Riddle, R. A., P. S. Dawson & D. F. Zirkle. 1986. An experimental test of the relationship between genetic variation and environmental variation in *Tribolium* flour beetles. *Genetics* **113**: 391-404.

Roff, D. 1992. The Evolution of Life Histories: Theory and Analysis. New York: Chapman and Hall. 535pp.

Scheltema, R. 1986. On dispersal and planktonic larvae of benthic marine invertebrates: an eclectic overview and summary of problems. *Bull. Mar. Sci.* **39**:290-322.

Sokal, R. R. & F. J. Rohlf. 1981. Biometry: the Principles and Practice of Statistics in Biological Research, 2nd ed. New York: W. H. Freeman. 859 pp.

Strathmann, R. R. 1985. Feeding and non-feeding larval development and life history evolution in marine invertebrates. *An. Rev. Ecol. Syst.* **16**:339-361.

_____. 1986. What controls the type of larval development? Summary statement for the evolution session. *Bull. Mar. Sci.* **39**(2): 616-622.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.* **25**:1-45.

Todd, C. D. 1979. The population ecology of *Onchidoris bilamellata* (L.) (Gastropoda: Nudibranchia). *J. exp. mar. Biol. Ecol.* **41**:213-255.

Todd, C. D. and J. N. Havenhand. 1988. Physiological ecology of *Adalaria proxima* (Alder *et* Hancock) and *Onchidoris muricata* (Müller) (Gastropoda: Nudibranchia). III. Energy budgets. *J. Exp. Mar. Biol. Ecol.* **118**:191-205.

Trapido-Rosenthal, H. G. and D. E. Morse. 1986. Availability of chemosensory receptors is down-regulated by habituation of larvae to a morphogenetic signal. *Proc. Natl. Acad. Sci.* **83**:7658-7662.

Travis, J. 1980. Genetic variation for larval specific growth rate in the frog *Hyla gratiosa*. *Growth* **44**: 167-181.

Vance, R. R. 1973A. On reproductive strategies in marine benthic invertebrates. *Am. Nat.* **107**:339-352.

_____. 1973B. More on reproductive strategies in marine benthic invertebrates. *Am. Nat.* **107**:353-361.

Wright, S. 1933. An analysis of variability in number of digits in an inbred strain of guinea pigs. *Genetics* **19**:506-536.

Personal Communications:

Dr. Francis Yeh — Dept. of Forest Sciences,
University of Alberta,
Edmonton, Alberta, Canada.

Chapter 6

Phenotypic and genetic correlation between life history traits and time of metamorphosis in the mollusc *Haminaea callidegenita*.

INTRODUCTION

Theories of life history evolution in benthic marine invertebrates are based on analyses of phenotypic traits with a primary focus on larval type. These data give us a description of variance available for selection in a particular population and have been used to generate predictions of the role of various traits in maximizing fitness (Mileikovsky 1971; Vance 1972 a&b; Caswell 1983; Strathmann 1985, 1986). For selection to produce evolutionary change, shifts in phenotypic expression must be passed on to the next generation (Falconer 1960). Change in one trait, both in degree and direction, is limited by the change possible in genetically correlated fitness traits. This may not be accurately reflected in estimates of phenotypic correlation which are often very small in populations near evolutionary equilibrium (Lande 1982). In benthic marine invertebrates, estimates of heritability and genetic correlations among life history traits have been largely ignored (except see Levin et al. 1991).

My objective in the present study was to determine potential genetic correlations among time of metamorphosis and other life history traits in the opisthobranch *Haminaea callidegenita*. This species has an unusual life history mode involving a dispersal polymorphism based on time of metamorphosis; the result is that both veligers and juveniles are released from every egg mass produced (Gibson & Chia 1989). Time of metamorphosis (inside the egg mass or after hatching) is highly variable both within and among clutches. Time of metamorphic competence is heritable and ability to delay metamorphosis is influenced by non-additive genetic effects. Additional reproductive traits are also heritable, including development time and clutch size (Chapter 5).

A sib analysis was used to test for genetic correlations among reproductive traits (interval between spawning events and clutch size), development time (embryonic and hatching periods) and time of metamorphosis of offspring (within the egg mass, after hatching and delayed metamorphosis). Genetic correlations among these traits would affect the degree to which evolutionary change in one life history trait is influenced by change in associated traits through pleiotropic effects or linkage disequilibrium (Lande 1982), and would indicate the degree to which phenotypic expression of traits and correlated traits are shaped by genetic or environmental effects (Becker 1975). Four types of correlations were

considered: 1) phenotypic correlation, which is the observed or measured association between two traits and represents the proportion of covariance subject to the action of selection; 2) genetic correlation through sire effects, indicating additive genetic covariance and estimating the underlying inherent genetic mechanism that directs and constrains the evolution of the traits involved; 3) genetic correlation through dam effects including additive and non-additive genetic effects (dominance and epistasis); and 4) environmental correlation which reflects the degree to which phenotypic expression is directed by the "environment" or everything excluded by the genetic model. In *Haminaea callidegenita*, many traits that appeared phenotypically to be independent were actually correlated genetically and environmentally. Intracapsular metamorphosis was positively correlated genetically with first metamorphosis after hatching, suggesting that selection for increased intracapsular metamorphosis may also increase the dispersal period for hatched veligers. Also, time of metamorphosis and ability to delay metamorphosis appeared phenotypically similar throughout the planktonic period, yet genetic differences suggested that they may respond differently to selection.

MATERIALS AND METHODS

A. Animal culture and breeding design

The experiment described in Chapter 5 was used for this study; therefore, procedures for animal culture and bioassays were described. Animals were mated in a hierarchical design allowing sib-analysis (Lawrence 1984; Falconer 1960), that was composed of groups consisting of one "male" bred with each of four "females" for a total of 10 males in 1991 and 11 in 1992 (Figure 5.2). *Haminaea* are simultaneous hermaphrodites and are capable of storing allosperm for several months (pers. obs.). To ensure known parentage, *Haminaea* were collected as small juveniles and raised in isolation at ambient conditions until reproductively mature. Every egg mass spawned by each "female" was collected (6-7 egg masses for most females) and measured for the traits listed below. Each trait was treated as a quantitative character, or as one showing continuous variation and determined by the combined action of several genes. For purposes of this analysis, one egg mass (or one clutch of eggs) was considered to represent one progeny.

Traits included female reproductive characteristics and time of metamorphosis of offspring. Traits are summarized here and a complete description of how each was quantified is provided in Chapter 5.

Traits included:

A. Reproductive traits (measured in 1991 and 1992):

INT spawning interval as the number of days between spawning events

HAT number of hatchlings per egg mass

EP length of embryonic period as the number of days from oviposition to hatching

HP length of the hatching periods also in days.

B. Time of metamorphosis, both within the egg mass (intracapsular), as:

JUV the percent of juvenile hatchlings/ egg mass (measured in 1991 and 1992)

and after hatching as the number of days until metamorphosis of hatched veligers in the presence of a metamorphic inducer (1992 only):

MF for the first (F) metamorphosis/ clutch (estimating minimum planktonic period)

MH metamorphosis of half (H) the hatched veligers/ clutch

MA metamorphosis of all (A) hatched veligers/ clutch (estimating maximum time of metamorphic competence)

C. The ability of hatched veligers to delay metamorphosis was determined as the number of days until metamorphosis in the absence of metamorphic inducer (1992 only):

DF for the first metamorphosis (F)/ clutch

DH metamorphosis of half (H) of the hatched veligers/ clutch

DA metamorphosis of all (A) hatched veligers/ clutch (estimating maximum planktonic period).

B. Statistical and genetic analysis

Statistical model

Statistical analyses were conducted with SPSS-X Release 3.0 for IBM MTS at the University of Alberta. Data were analyzed with nested ANOVA (UANOVA procedure) with males nested within years and females nested within males and years. Males and females were designated as random (Lawrence 1984). As female size can influence the expression of certain traits (egg mass size and the duration of the hatching period; Chapter 5), female wet weight (mg) at the onset of the spawning period was incorporated in the model statement as:

model = wt + year + wt * year + male (year) + wt * male (year) + female (wt, year, male) + residual .

The results from this ANOVA were used in the statistical model

$$X_{ijk} = \mu + S_i + D_{ij} + E_{ijk}$$

where X_{ijk} = the record of the k^{th} offspring of the j^{th} female mated to the i^{th} male,

μ = common mean, S_i = effect of i^{th} male, D_{ij} = effect of the j^{th} female mated to the i^{th} male, and E_{ijk} = uncontrolled environmental and genetic deviations attributable to offspring. This model assumes that individuals are chosen at random from a reference population that is a non-inbred random mating population (Falconer 1960).

Analysis of individual traits

The phenotypic expression of an individual trait can be partitioned into variance components attributable to each parent, and subsequently decomposed into the relative contributions of genetic and environmental effects that determine the observed expression (Chapter 5). Variance components are partitioned as follows:

σ^2_s = sire components, or the differences among offspring of particular sires, which include additive genetic variance;

σ^2_d = dam components, or differences among offspring of dams mated to the same sire, including additive and non-additive (dominance, epistasis) genetic variance;

σ^2_w = progeny components, or differences between individual offspring of the same dam, including additive genetic variance, non-additive genetic variance, and environmental effects;

σ^2_t = phenotypic variance, or the sum of the variance attributable to each of the three components ($\sigma^2_t = \sigma^2_s + \sigma^2_d + \sigma^2_w$).

These components can be used to estimate heritability as

$$h^2 = 4 (\sigma_s^2) / \sigma^2_t$$

or the genetic variance attributable to either sire (narrow sense heritability) or dam (broad sense heritability) over the total variance observed (Becker 1975). The heritability of individual traits may not be detected if the trait of interest is negatively associated with a second trait (Falconer 1960). The underlying genetic basis for these traits may be uncovered by examining the degree to which these traits are correlated through additive genetic covariance.

Correlations among traits

The covariance between two traits was estimated using the sum of squares cross products calculated by UANOVA. The statistical derivations of estimates of covariance are as follows:

Source	df	Mean Cross Products	Estimation of Mean Cross Products
Sires	s-1	MCP_s	$cov_w + k^2\ cov_d + k^3\ cov_s$
Dams	d-s	MCP_d	$cov_w + k^1\ cov_d$
Progeny	n-d	MCP_w	cov_w

where s = sires, d = dams, w = progeny, n = number of observations, $k^1 = k^2$ = number of progeny/ dam and k^3 = number of progeny/ sire. The genetic model underlying the components of covariance is the same as for the variance components of individual traits, so that:

cov_s = 1/4 additive genetic covariance;

cov_d = 1/4 additive genetic covariance, 1/4 covariance due to dominance effects and their interactions, and;

cov_w = 1/2 additive genetic covariance, 1/4 dominance covariance, their interactions, and all of the environmental covariance (Becker 1975).

Correlations were determined as the ratio of covariance between traits x and y over the standard deviations of each as an individual trait (Becker 1975). Phenotypic correlation is the association between two characters that can be directly observed in a population and is calculated as:

$$r_p = \frac{cov_w + cov_s + cov_d}{\sqrt{(\sigma^2_{w(x)} + \sigma^2_{s(x)} + \sigma^2_{d(x)})}\ \sqrt{(\sigma^2_{w(y)} + \sigma^2_{s(y)} + \sigma^2_{d(y)})}}$$

where cov is the covariance and σ^2 the variance component of traits x and y for progeny (w), sires (s) and dams (d). This estimate therefore includes the total variance as produced by sire, dam, and progeny effects for traits x and y as well as all genetic and environmental effects and conceptually can be abbreviated as

$$r_p = h^2 + e^2.$$

where h^2 = heritability and e^2 = environmental effects.

Genetic correlations occur primarily through pleiotropy which is the effect of a single gene on two or more characters (Falconer 1960). Some genes will have a positive effect on both traits and will result in a positive correlation; others will have a positive effect on one trait and a negative effect on the other, resulting in a negative or un-detectable correlation. Correlations were examined for all traits observed, even if heritability was not detected in individual traits to allow for the possibility of traits that might be "hidden" in this way. Genetic correlations through the sire components are estimated as

$$r_{g(s)} = \frac{cov_s}{\sqrt{\sigma^2_{s(x)}}\ \sqrt{\sigma^2_{s(y)}}}.$$

Therefore, $r_{g(s)}$ includes additive genetic covariance and indicates if the correlation is subject to evolutionary change through selection, or, the extent to which two measurements reflect what is genetically the same character (Falconer 1960). Genetic correlations through dam components are estimated in the same way using dam covariance and variance components; these correlations contain non-additive genetic effects as well.

Environmental correlations include covariance due to common environment and maternal effects and exclude additive and non-additive genetic variance. They are estimated as the covariance attributable to the progeny minus the covariance attributable to sires and dams and are derived as:

$$r_e = \frac{cov_w - cov_s - cov_d}{\sqrt{(\sigma^2_{w(x)} - \sigma^2_{s(x)} - \sigma^2_{d(x)})}\ \sqrt{(\sigma^2_{w(y)} - \sigma^2_{s(y)} - \sigma^2_{d(y)})}}$$

Standard errors were calculated as by Becker (1975) for a hierarchical design as S.E.(r) = $\sqrt{\text{var r}}$. Significance of the correlation coefficients was determined using the t-distribution (Sokal & Rohlf 1981). Degrees of freedom associated with error estimates are given in each table. Prior to statistical analysis, the phenotypic expression of each trait was compared with other traits using a scatterplot in the analysis to ensure that correlations reflect linear relationships rather than a more complex function that would not be detected by the statistical model outlined here.

RESULTS

A. Correlations among life history traits and intracapsular metamorphosis

Phenotypic values and heritability estimates for life history traits were described in Chapter 5 and are summarized in Table 6.1. Clutch size was heritable through sires indicating additive genetic variance. Spawning interval, hatching period and egg mass size showed genetic variance through dams which was inflated through non-additive genetic effects. Onset of metamorphic competence within the egg mass (determined as the percent of juvenile hatchlings) was heritable through both sires and dams and was only slightly influenced by non-additive genetic effects (Table 6.1).

Phenotypic correlations are illustrated in Figure 6.1A using a 3-dimensional plot in which the x and y axes represent traits x and y, and the z axis the degree to which they are correlated (positively or negatively). Correlations are repeated in Table 6.2 (including error estimates and significance levels) to show phenotypic (upper right triangle), genetic (sires and dams) and environmental correlations (all three in lower left triangle, listed from top to bottom).

The phenotypic expressions of these reproductive traits were remarkable primarily for their apparent lack of correlation (Figure 6.1A). The one exception was the strong positive correlation between hatching period (HP) and number hatched (HAT; $r_p = 0.62$) that showed that larger egg masses also took longer to hatch. Genetic correlations revealed that these two traits were highly correlated through the genetic contributions of both sire and dam ($r_{g(s)} = 0.78$ and $r_{g(d)} = 0.79$; Table 6.2; Figure 6.1B). The apparent equal

contributions from both parents indicated that these traits were largely determined by the same additive genetic mechanism and that expression through dam effects was not further modified by non-additive genetic effects. Environmental components of covariance also supported the positive correlation between HP and HAT (Figure 6.1C).

Females that spawned less frequently also produced larger egg masses although this correlation was very low (INT and HAT, $r_p = 0.11$; Figure 6.1A); this correlation was most strongly influenced by environmental effects ($r_e = 0.27$; Figure 6.1C) and the genetic correlations were not significant. Larger egg masses also had a longer embryonic period (HAT and EP, $r_p = 0.14$; Figure 6.1A). The sire correlation for EP and HAT was significant ($r_{g(s)} = 0.69$) but the dam correlation was not, suggesting non-additive genetic mechanisms may be countering additive genetic effects, possibly through dominance of one allele. The environmental effect was also not significant (Table 6.2). Embryonic period was genetically correlated with hatching period ($r_{g(s)} = 0.57$); dam and environmental correlations were not significant which may have resulted in the lack of phenotypic correlation (Figure 6.2).

Egg masses releasing a higher percent of juveniles (JUV) also had slightly longer embryonic periods (EP, $r_p = 0.11$) and contained slightly fewer larvae (HAT, $r_p = -0.10$; Figure 6.1A). Despite the small phenotypic correlations, the genetic covariance between JUV and other traits was often large and positive (Figure 6.1B). JUV was positively correlated through additive genetic covariance with development time within the egg mass (EP, $r_{g(s)} = 0.46$, and HP, $r_{g(s)} = 0.61$) through sire components, and less strongly (not significant) with HAT ($r_{g(s)} = 0.33$; Figure 6.1B). Estimates from the dam components were not significant (Table 6.2) indicating that the genetic covariance between these traits was modified by non-additive effects. Environmental correlations showed strong negative correlations between JUV and both HP and HAT (Figure 6.1C). Again, the combination of sire, dam, and environmental effects may account for the observed lack of phenotypic correlation.

B. Time of extracapsular metamorphosis and ability to delay metamorphosis
Correlations with reproductive traits

In 1992, the time of metamorphosis after hatching was considered in addition to the reproductive traits discussed above for both years. Hatched veligers became competent to metamorphose within 13 d of hatching; time of competence was heritable and strongly influenced by non-additive genetic effects late in the planktonic period (included in the estimate of broad-sense heritability; Table 6.1). Metamorphosis could be delayed an additional week. The ability to delay showed no heritable components through sire effects

but was influenced by non-additive effects in the mid-planktonic period (Table 6.1). Table 6.3 contains phenotypic, genetic and environmental correlations for 1992.

Reproductive traits were described in the following analysis primarily in association with time of metamorphosis and the ability of larvae to delay metamorphosis. In some cases, the correlations among reproductive traits in 1992 differed from those observed when both years were considered together. The primary difference arose from the influence of environment on embryonic period (Table 6.3) which showed significant covariance with HP and JUV but not with HAT, as was observed in 1991. Genetic correlations between embryonic period and HP or HAT were similar in direction in 1992 to observations obtained when both years were compared, but were not significant. The genetic correlation between EP and JUV was not significant in 1992 (Table 6.3). Despite this difference, both years were considered in the analysis described above to increase sample size. Year differences were partitioned as the major factor in the ANOVA.

Phenotypic correlations were not detected for reproductive traits and time of metamorphosis after hatching, except for a significant correlation between EP and DH ($r_p = 0.25$; Table 6.3). Correlations from additive genetic and non-additive genetic covariance were also not significant, although in some cases the correlations were relatively large and showed opposite effects. For example, genetic correlations between HP and DF ($r_{g(s)} = 0.28$, $r_{g(d)} = -0.25$) and HP and DH ($r_{g(s)} = 0.37$ and $r_{g(d)} = -0.23$), although not significant, may be indicative of genetic covariance that would be revealed in a larger study.

Environmental correlations were significant for several trait pairs, such as clutch size and time of metamorphosis after hatching (MF, $r_e = -0.64$, MH, $r_e = -0.19$) or ability to delay (DH, $r_e = -0.22$, DA, $r_e = -0.26$; Table 6.3), suggesting that environmental effects cause hatchlings from larger clutches to reach metamorphic competence sooner and have a decreased ability to delay metamorphosis. These effects were not reflected in phenotypic correlations, probably because of sire effects which were insignificant statistically but were large enough to suggest that additive genetic covariance may be creating the opposite effect in terms of time of metamorphosis for most of the planktonic period (e.g., HAT and MF, $r_{g(s)} = 0.50$, MH, $r_{g(s)} = 0.39$; Table 6.3).

Time of metamorphic competence

Phenotypic correlations showed that veligers hatching from egg masses with a high percent of intracapsular metamorphosis (JUV) took longer to begin metamorphosis (MF, $r_p = 0.31$; Figure 6.2A). In contrast, the maximum metamorphic period in these clutches was relatively short (MA, $r_p = -0.28$). This pattern was strongly supported by dam

correlations, and by environmental effects late in the planktonic period; correlations from sire effects were not significant but were of the same direction (Figure 6.2B&C, Table 6.3).

Time of metamorphic competence throughout the planktonic phase (i.e., after hatching) followed the same phenotypic pattern in that clutches with the longest period before the first metamorphosis of a hatched veliger also showed longer planktonic periods for most veligers/ clutch (MF and MH, $r_p = 0.44$); correlation with maximum planktonic period was insignificant but in the same direction as occurred in the above correlations (MF and MA, $r_p = -0.18$; Figure 6.2A). Dam covariance was similar in direction (Table 6.3) suggesting that the time metamorphosis occurred was largely determined by non-additive genetic effects (Figure 6.2B). Environmental correlations with MF were positive throughout the planktonic period (MH, MA) indicating time of metamorphosis outside the egg mass may be extended by non-genetic effects (Figure 6.2C).

Ability to delay metamorphosis

Clutches with a high ability to delay metamorphosis immediately after hatching also showed a longer period of delay for most larvae (DF and DH, $r_p = 0.62$); correlation with the maximum delay period was not significant (Table 6.3). Phenotypic covariance was influenced by both genetic and environmental effects early in the planktonic period (DF and DH, $r_{g(s)} = 0.85$, $r_{g(d)} = 0.76$, $r_e = 0.30$). Clutches with a high ability to delay for most veligers also had the greatest ability to delay overall (DH, DA, $r_p = 0.41$). Later in the delay period, non-additive genetic (DH, DA, $r_{g(d)} = 0.47$) and environmental covariance ($r_e = 0.63$) had strong positive effects; sire effects not significant but were in the same direction (Table 6.3).

Correlations between competence and ability to delay metamorphosis

Phenotypic correlations between percent of juveniles (JUV) and the ability to delay metamorphosis (Figure 6.3A) were positive early in the planktonic period ($r_p = 0.27$), as occurred for time of competence (Figure 6.2A). Environmental correlations were positive at the beginning (JUV and DF, $r_e = 0.25$) and negative at the end of the planktonic period (JUV and DA, $r_e = -0.30$; Figure 6.3), or, occurred in the same direction as for time of competence. Sire effects were not significant but were positive throughout the planktonic period (JUV and DF, $r_{g(s)} = 0.42$, JUV and DA, $r_{g(s)} = 0.48$; Figure 6.3B).

Within the planktonic period, early onset of metamorphic competence (MF) showed no phenotypic correlation with the initial ability to delay metamorphosis (DF; Table 6.3). However, clutches with a rapid onset of competence showed a decreasing ability to delay

metamorphosis at the end of the planktonic period (MF and DA, r_p = -0.28; Table 6.3). Environmental correlations indicated clutches with rapid onset of competence had an increased initial ability to delay (r_e = 0.26) but this effect changed at the end of the planktonic period (r_e = -0.43). Additive genetic covariance was not significant but estimates were negative throughout the planktonic period (e.g., MF and DF, $r_{g(s)}$ = -0.41). Veligers hatching from egg masses with a long absolute period before all become competent (MA) were not phenotypically correlated with maximum ability to delay (DA; Table 6.3).

DISCUSSION

Selection acts on phenotypic variance to change the immediate expression of that trait. Evolutionary change only occurs if the trait is heritable and only within limits defined by genetic covariance with other traits (Falconer 1960). These constraints may not be accurately reflected in phenotypic correlations which are often small for populations in equilibrium because of contrasting effects of large genetic and environmental correlations (Lande 1982). Comparison of the phenotypic correlations between the reproductive traits examined here suggested that most are independent although a closer examination of genetic (additive and non-additive) and environmental correlations revealed that this was not always the case. In this discussion, I have suggested possible mechanisms by which genetics and environment may act to determine the observed phenotypic expression. Correlations do not represent causal relationships and mechanisms are proposed only as further avenues of study.

Genetic and environmental correlations among these traits often differed in degree and direction, creating phenotypic correlations that were very low. A large difference in r_g and r_e, particularly a difference in sign, indicates that additive genetic and environmental sources of covariance may be affecting characters through different physiological mechanisms (Falconer 1960). An example is the correlation between the percent of juvenile hatchlings (JUV) and hatching period (HP; r_p = -0.001, $r_{g(s)}$ = 0.61, r_e = -0.79; Figure 6.1). Although genetically similar, time of intracapsular metamorphosis and time of hatching may be achieved by separate physiological or ontogenetic pathways. This may allow larvae that develop more slowly to the point of hatching to achieve metamorphic competence at the same chronological time as their earlier hatching siblings. Therefore, additive genetic covariance would reflect a higher incidence of juveniles hatching from egg masses with a longer hatching period. The negative environmental correlation between JUV and HP suggests the involvement of other mechanisms that promote the opposite

effect. Possibilities include an independent release of a hatching enzyme causing earlier release of siblings from the same egg mass than determined by the above mechanism, the physical disruption of the egg mass by early hatchlings causing pre-mature release of siblings, or the involvement of traits other than those examined in this study. It is interesting that the process is much more complex than a straightforward phenomenon of egg masses with longer hatching periods releasing more juveniles simply because they represent a later ontogenetic stage.

The percent of juvenile hatchlings may be a trait that is sensitive to evolutionary change because it is highly heritable (h^2_S=0.76), shows little influence of non-additive genetic variance, and also is responsive to short term changes in parental environment (Chapter 5). Time of metamorphosis was correlated throughout the metamorphic period (pre- and post-hatching) such that clutches with a high rate of intracapsular metamorphosis also had a longer planktonic period for most hatched veligers (JUV and MF, $r_p = 0.31$) but a decreased maximum planktonic period (JUV and MA, $r_p = -0.28$). These correlations were primarily affected by non-additive genetic covariance; sire effects were in the same direction but were not significant.

The similarity in direction of additive and non-additive covariance in time of metamorphosis suggests that selection for an increase in the percent of juveniles may also increase the duration of the planktonic phase of most of the remaining veliger hatchlings. A possible result may be that *Haminaea callidegenita* would continue to exploit their poecilogonous life history pattern despite potential selection for early metamorphosis and more juvenile hatchlings. Most progeny would immediately recruit into the parental habitat but the majority of dispersive offspring (MF, MH) would have an even longer period in which to intercept an acceptable, and perhaps more distant, habitat. A negative genetic correlation between early competence of hatched veligers and maximum time of competence in the plankton (MF and MA, $r_{g(s)} = -0.34$, $r_{g(d)} = -0.55$) may restrict the maximum planktonic period. A short maximum dispersal period (MA) may be advantageous to *Haminaea* because it decreases the risks associated with a long planktonic period, such as increased advection from favorable habitats (Palmer & Strathmann 1981) and predation of vulnerable larvae (Rumrill 1986). However, it is not possible to predict long term effects of selection on these traits as estimates of genetic correlations are strongly influenced by gene-frequency and hence may change within a few generations (Lande & Arnold 1983).

Time of metamorphic competence and ability to delay metamorphosis show the same phenotypic pattern but appear to differ genetically; therefore, they may respond differently to selection. Early competence (JUV) and time of metamorphosis after hatching (MF, MA) were correlated through non-additive genetic covariance; correlations through

additive genetic covariance were not significant. Early competence and ability to delay (DF, DA) were not influenced by non-additive genetic covariance; although sire estimates were not significant, they were consistently high and positive throughout the metamorphic period suggesting their potential importance ($r_{g(s)}$ for JUV and DF = 0.42, for DH = 0.35, for DA = 0.48). In this case, lack of significance may reflect the small number of sires used in the study (n=11). These correlations suggest that different genetic mechanisms may determine onset of competence and the ability to delay.

Environmental correlations were similar in direction for early competence (JUV) and both competence after hatching and the ability to delay metamorphosis suggesting that environmental mechanisms have a similar effect on both time of competence and the ability to delay. Veligers released from egg masses with a high percent of juvenile hatchlings may be genetically able to delay metamorphosis longer, but environmental constraints on their development may trigger a pre-determined threshold instigating metamorphosis in the absence of the expected cue (e.g., the metamorphic inducer). The source of the environmental variance is not known but as these are non-feeding larvae, a possibility is that larvae reach a state of yolk depletion where larvae must metamorphose or risk not having the energetic reserves to successfully complete the transformation from larva to juvenile.

Several pairs of traits were most strongly influenced by environmental effects. Environmental covariance was positive for fecundity such that females that spawned at greater intervals also produced larger clutches (r_e = 0.64). This may reflect maternal effects, such as female size or reproductive condition, that may allow females that spawn less frequently to put more effort into each spawning event. Environmental correlations also showed that larvae hatching from larger clutches had a decreased maximum ability to delay metamorphosis (DA, r_e = -0.26). This suggests the potential for an energetic trade-off between number of eggs produced and organic content/ egg. An influence of egg size on ability to delay metamorphosis was not detected (Chapter 5) but egg size does not necessarily reflect organic content in all taxa (McEdward & Coulter 1987). Genetic correlation between clutch size and ability to delay was not detected; therefore, the evolutionary significance of this correlation, if any, is not apparent.

Another consequence of deviations caused by environment was the difference in expression of some traits between years. The environmental correlations that were most affected included embryonic period such that correlations observed in both years (EP and HAT) were not detected in 1992, but additional correlations (EP and HP, JUV) were found in 1992. Phenotypic correlations, although small, were also modified presumably because of the influence of environment. The strong effect of environmental influence on

correlations with embryonic period was interesting because embryonic period had the most conservative expression of all individual traits considered in that there was little overall variance, the mean expression did not differ between the two years, and expression was not influenced by female size (Chapter 5). Genetic correlations involving embryonic period also differed between the two years such that correlations detected in both years (EP and HP, HAT, JUV) were not significant in 1992, although two of these correlations were in the same direction (HP and HAT). This may be a result of the small number of sires used in the 1992 analysis, but may additionally reflect genotype-environment interactions which may produce different estimates of genetic correlation in different environments (Falconer 1960, Via & Lande 1985). Although the same "common garden" was used to maintain adults in both years, many uncontrolled environmental factors may have differed between the two years with unknown effects (e.g., the effects of winter conditions on juveniles, ambient water temperature, or diatom growth within the cages).

Conclusion

This study demonstrated that the seemingly independent expression of several life history traits was actually based on a complex interaction between genetic and environmental components, potentially active through opposing mechanisms. Year differences were also noted emphasizing the importance of consideration of traits in more than year. That the phenotypic expression of most of these traits was fairly plastic is supported by the large effect of environmentally mediated covariance, and the influence of the reproductive condition of females, such as female size and changes occurring throughout the spawning season (Chapter 5). Time of metamorphosis was based on several factors. It appeared to be polygenic and may be variously influenced by dominance and environmental effects. Similar observations have been made on life history traits in other species (Falconer 1960; Hegmann & Dingle 1982; Kelly 1993) and similar correlations are undoubtedly widespread in benthic marine invertebrates. Evolutionary theories and models considering the advantages attributed to various life history "strategies" must also consider whether evolutionary change is likely in the traits considered, through estimates of heritability and genetic correlation (Roff 1992).

Table 6.1: Overall means (± standard deviations) and heritabilities (± standard errors) calculated for sire (h^2_S) and dam (h^2_d) variance components for life history parameters of *Haminaea callidegenita* . Traits are defined in the text. n = number of egg masses examined for each trait.

Trait	Abbreviation	n	Mean	h^2_S	h^2_d
Spawn interval (d)	INT	468	6.97±0.26	0	0.53±0.16
Embryonic period (d)	EP	468	14.35±0.07	0.08±0.06	0
Hatching period (d)	HP	468	4.91±0.10	0.19±0.21	0.98±0.19
Egg mass size	HAT	468	345.40±6.43	0.34±0.06	1.11±0.18
Percentage of juvenile hatchlings	JUV	468	88.71±12.26	0.76±0.45	0.85±0.25
Time of metamorphic competence					
1st metamorphosis	MIF	137	3.66±0.13	0.35±0.21	0
50% metamorphosed	MIH	137	5.95±0.18	0.15±0.19	0
100% metamorphosed	MIA	137	13.65±0.55	0.22±0.33	0.76±0.31
Ability to delay metamorphosis					
1st metamorphosis	SWF	123	4.78±0.35	0	0
50% metamorphosed	SWH	123	9.22±0.49	0.06±0.28	0.66±0.37
100% metamorphosed	SWA	123	20.63±0.76	0	0.01±0.29

Table 6.2: Correlations of *Haminaea callidegenita* life history traits calculated for 1991 and 1992. Correlations are listed for each trait such that the upper right triangle of the table contains phenotypic correlations (r_p) between traits x and y and the lower left triangle contains genotypic correlation for sires±S.E.($r_{g(s)}$; top value), genotypic correlation for dams±S.E. ($r_{g(d)}$; mid value) and environmental correlation (r_e; bottom value). Self correlations (r=1) are included along the center line. Traits listed are: INT- interval between spawning events (d); EP- embryonic period (d); HP- hatching period (d); HAT- number of hatchlings/ egg mass; and JUV- the percent of hatchlings released as juveniles/ egg mass. df = 18 for sires, 48 for dams, 400 for progeny. **p<0.01, *p<0.05

	INT	EP	HP	HAT	JUV
INT	1	0.09	0.07	* 0.11	0.09
EP	0.15±0.34 0.15±0.11 0.08	1	0.00	** 0.14	** 0.11
HP	-0.06±0.39 -0.05±0.03 ** 0.21	** 0.57±0.47 -0.14±0.13 -0.08	1	** 0.62	-0.001
HAT	-0.11±0.74 -0.03±0.01 ** 0.27	** 0.69±0.48 -0.14±0.04 ** 0.15	** 0.78±0.62 ** 0.79±0.09 ** 0.40	1	* -0.10
JUV	0.26±0.43 0.06±0.07 0.02	* 0.46±0.41 0.21±0.35 -0.06	** 0.61±0.51 -0.14±0.07 ** -0.79	0.33±0.22 -0.16±0.07 ** -0.64	1

Table 6.3: Correlations for *Haminaea callidegenita* life history traits and time of metamorphosis for 1992. Correlations are listed for each trait such that upper right triangle of the table contains phenotypic correlations between traits x and y (r_p) and the lower left triangle contains genotypic correlations for sires ± S.E.($r_{g(s)}$; top value) , genotypic correlations for dams ± S.E. ($r_{g(d)}$; mid value) , and environmental correlations (r_e; lower value). Traits included are: INT interval between spawning events, EP embryonic period (d), HP hatching period (d), HAT number of hatchlings/ egg mass, JUV percentage of juvenile hatchlings/ egg mass, MF time of metamorphosis in the presence of metamorphic inducer by the first veliger/ clutch, MH time of metamorphosis of 50% hatched veligers/ clutch, MA time of metamorphosis of all veligers/ clutch, DF time metamorphosis can be delayed by the first, DH by 50% and DA by all hatched veligers/ clutch. df = 10 for sires, 22 for dams, and 77 for progeny. **p<0.01, *p<0.05

	INT	EP	HP	HAT	JUV	MF	MH	MA	DF	DH	DA
INT	1	-0.10	0.16	-0.01	0.07	0.04	0.15	-0.16	0.18	0.02	-0.04
EP	–0.09 ± 0.43 0.25 ± 0.14 * –0.23	1	* -0.19	0.07	-0.16	0.00	-0.06	0.01	0.15	** 0.25	0.12
HP	0.11 ± 0.76 0.05 ± 0.02 **0.28	0.16 ± 0.14 –0.38 ± 0.26 **–0.31	1	** 0.53	-0.10	-0.02	0.13	-0.01	0.06	0.01	0.00
HAT	–0.26 ± 0.62 –0.08 ± 0.07 *0.23	0.38 ± 0.31 –0.15 ± 0.08 –0.03	*0.64 ± 0.42 **0.52 ± 0.15 **0.41	1	-0.17	0.00	0.00	0.15	0.05	0.03	-0.11
JUV	–0.11 ± 0.55 **0.58 ± 0.46 –0.13	–0.08 ± 0.05 –0.07 ± 0.04 * –0.24	*0.67 ± 0.47 –0.19 ± 0.08 ** –0.74	0.52 ± 0.49 –0.15 ± 0.06 ** –0.85	1	** 0.31	0.12	** -0.28	** 0.27	0.10	-0.07
MF	–0.17 ± 0.86 0.35 ± 0.75 0.05	0.35 ± 0.39 0.24 ± 0.24 * –0.22	0.15 ± 0.20 –0.18 ± 0.70 –0.14	0.50 ± 0.47 0.10 ± 0.06 **–0.64	0.14 ± 0.15 **0.57 ± 0.26 **0.32	1	** 0.44	-0.18	0.02	-0.16	** -0.28
MH	–0.37 ± 1.93 0.25 ± 0.15 **0.27	0.35 ± 0.38 –0.03 ± 0.03 –0.16	0.13 ± 0.59 0.24 ± 0.31 0.11	0.39 ± 0.37 –0.03 ± 0.09 * –0.19	0.04 ± 0.11 0.15 ± 0.17 0.13	0.05 ± 0.09 0.38 ± 0.06 **0.65	1	0.18	0.08	0.05	-0.15
MA	–0.33 ± 2.77 –0.25 ± 0.51 0.02	0.06 ± 0.16 –0.11 ± 0.21 0.09	–0.24 ± 0.49 0.36 ± 0.31 –0.11	0.16 ± 1.02 0.19 ± 0.44 0.02	–0.20 ± 0.15 **–0.45 ± 0.37 –0.16	–0.34 ± 1.58 **–0.55 ± 0.42 **0.29	*0.60 ± 0.61 0.04 ± 0.02 0.07	1	0.05	0.14	-0.11
DF	–0.14 ± 0.68 0.29 ± 0.23 *0.24	–0.16 ± 0.15 0.25 ± 0.24 0.19	0.28 ± 0.22 –0.25 ± 0.21 0.14	0.24 ± 0.22 –0.03 ± 0.08 –0.01	0.42 ± 0.35 0.21 ± 0.11 *0.25	–0.41 ± 0.35 0.05 ± 0.06 **0.26	0.16 ± 0.24 0.06 ± 0.11 0.07	0.06 ± 0.12 –0.27 ± 0.15 **0.51	1	** 0.62	0.13
DH	–0.23 ± 0.21 0.28 ± 0.31 –0.02	0.03 ± 0.04 0.38 ± 0.23 **0.36	0.37 ± 0.38 –0.23 ± 0.48 ** –0.30	0.17 ± 0.09 0.02 ± 0.11 * –0.22	0.35 ± 0.32 0.17 ± 0.68 ** –0.26	–0.54 ± 0.43 0.13 ± 0.10 0.03	0.46 ± 0.54 –0.07 ± 0.28 –0.10	0.27 ± 0.67 *–0.41 ± 0.14 ** 1.23	**0.83 ± 0.73 **0.76 ± 0.22 **0.30	1	**0.41
DA	0.00 ± 0.00 0.02 ± 0.01 –0.07	–0.01 ± 0.04 0.09 ± 0.08 0.15	0.03 ± 0.07 –0.11 ± 0.31 0.05	–0.17 ± 0.34 0.09 ± 0.06 ** –0.26	0.48 ± 0.44 –0.02 ± 0.01 ** –0.30	–0.24 ± 0.25 0.01 ± 0.01 ** –0.43	–0.28 ± 0.56 –0.32 ± 0.48 –0.06	–0.20 ± 0.25 –0.30 ± 0.23 0.09	0.35 ± 0.28 0.02 ± 0.02 0.13	0.23 ± 0.31 **0.47 ± 0.14 **0.63	1

Figure 6.1. Correlations among life history traits in *Haminaea callidegenita*, including A phenotypic, B additive genetic (through sires), and C environmental correlations. Traits are : INT = spawning interval; EP = embryonic period; HP = hatching period; HAT = number hatched; and JUV = proportion of juveniles for each egg mass. Self correlations have been set to 0.

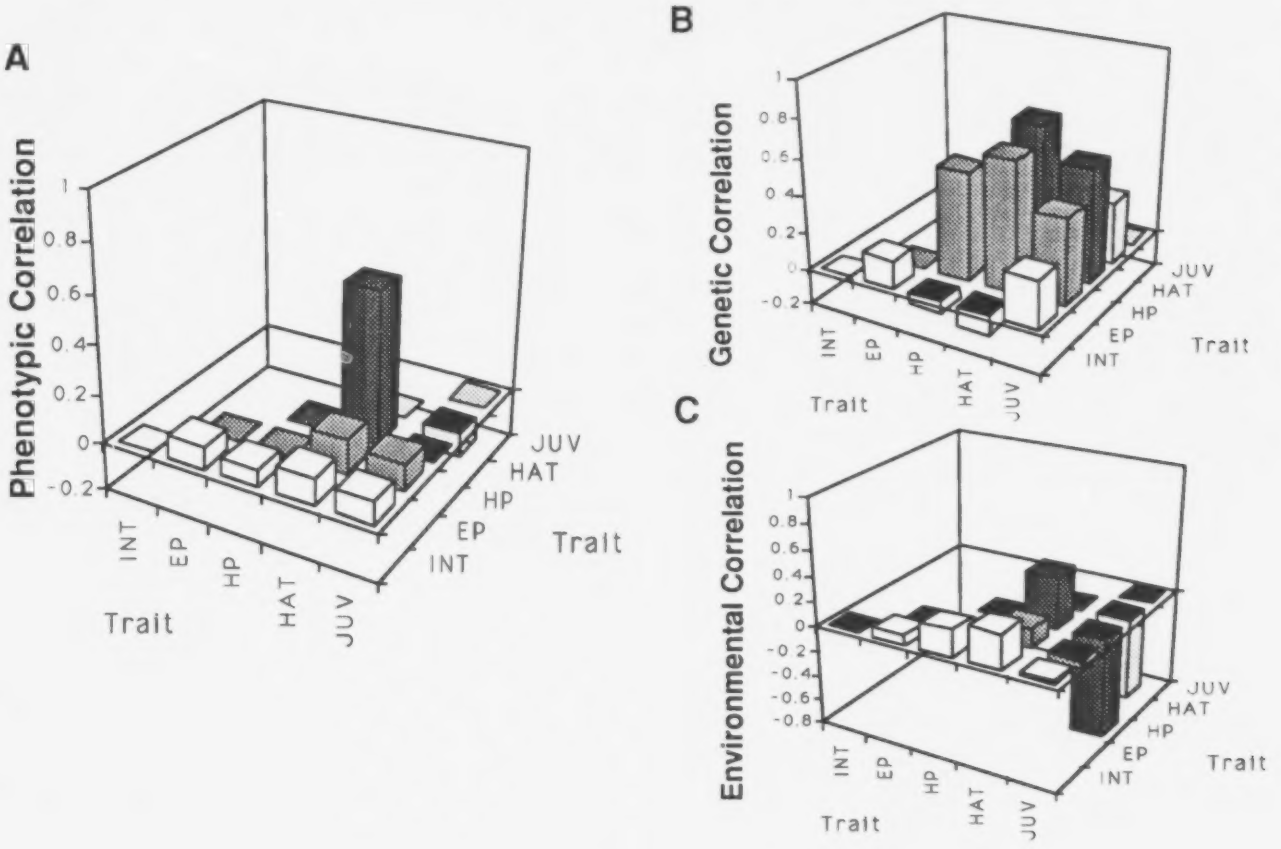


Figure 6.2. Correlations for time of metamorphosis in *Haminaea callidegenita*, including A phenotypic, B additive genetic (through sires), and C environmental correlations. Traits are: JUV = the proportion of juveniles; MF = time of first metamorphosis, MH = of half, and MA = of all hatched veligers/ clutch in the presence of a metamorphic inducer.

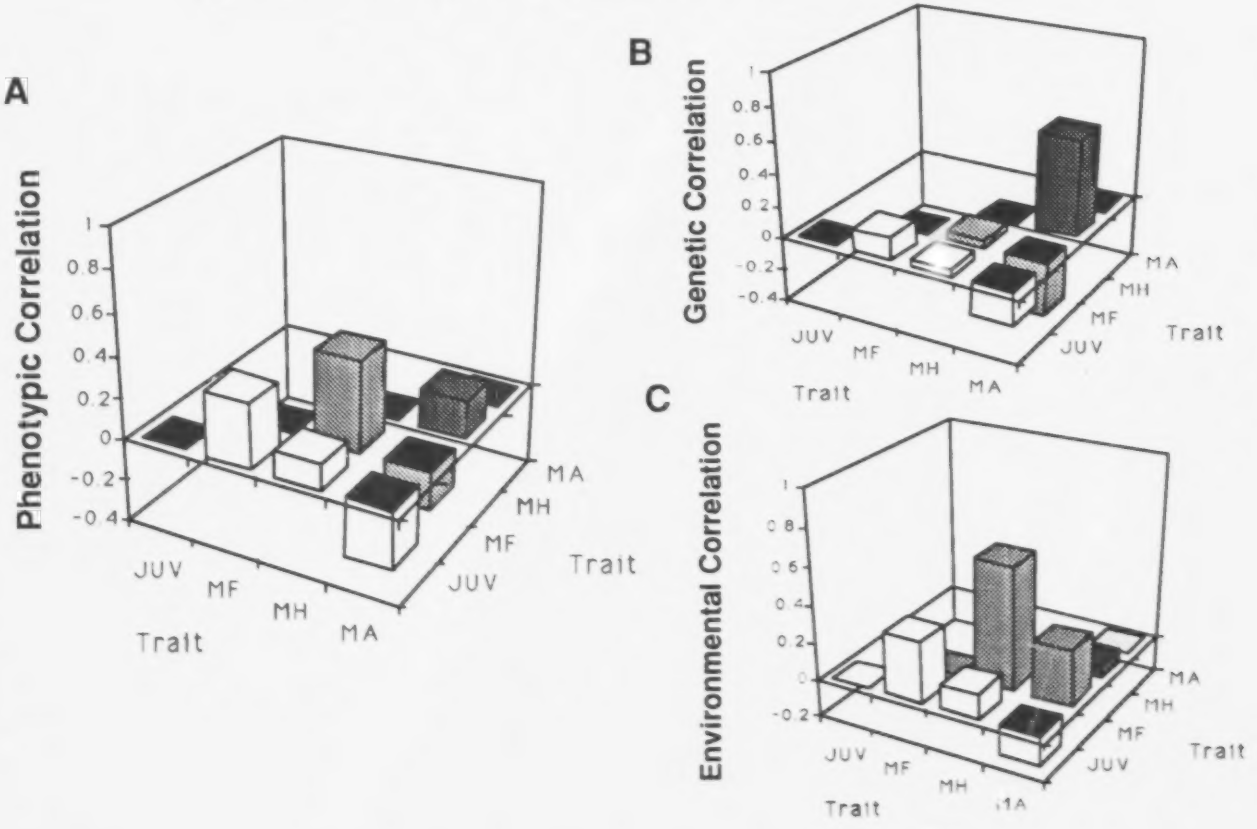
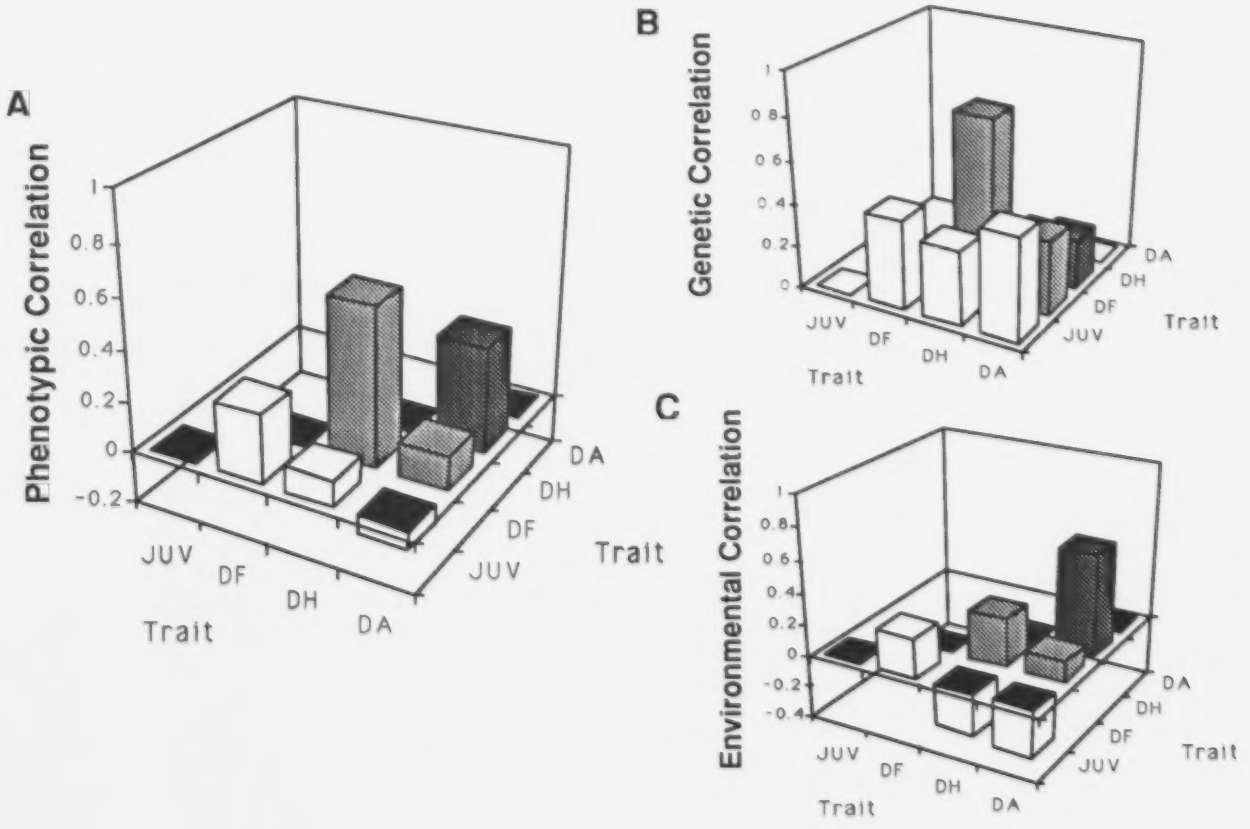


Figure 6.3. Correlations for ability to delay metamorphosis in *Haminaea callidegenita*, including A phenotypic, B additive genetic (through sires), and C environmental correlations. Traits are: JUV = proportion of juvenile hatchlings; DF = time of first metamorphosis, DH = of half, and DA = of all hatched veligers/ clutch in absence of metamorphic inducer.



Literature Cited

Becker, W. A. 1975. Manual of Quantitative Genetics, 4th ed. Pullman, WA: Academic Enterprises. 190 pp.

Caswell, H. 1983. Phenotypic plasticity in life history traits: demographic effects and evolutionary consequences. *Am. Zool.* **23**:35-46.

Falconer, DS. 1960. Introduction to Quantitative Genetics. New York: Ronald Press Co. 365 pp.

Gibson, G. D. & F.-S. Chia. 1989. Developmental variability (pelagic and benthic) in *Haminaea callidegenita* (Opisthobranchia: Cephalaspidea) is influences by egg mass jelly. *Biol. Bull.* **176**:103-110.

Hegmann, J. P. & H. Dingle. 1982. Phenotypic and genetic covariance structure in milkweed bug life history traits. *In* Evolution and Genetics of Life Histories *Ed.*: H. Dingle and J. P. Hegmann. New York: Springer-Verlag. pp 177-185

Kelly, C. A. 1993. Quantitative genetics of size and phenology of life-history traits in *Chamaecrista fasciculata*. Evolution **47**(1): 88-97.

Lande, R. 1982. A quantitative genetic theory of life history evolution. *Ecology* **63**(3):607-615.

Lande, R. and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210-1226.

Lawrence, M. J. 1984. The genetical analysis of ecological traits. *In*: Evolutionary Ecology: the 23rd Symposium of the British Ecological Society Leeds 1982. *Ed.*: B. Shorrocks. Oxford: Blackwell Scientific Publications. pp 27-63.

Levin, L. A., J. Zhu, and E. Creed. 1991. The genetic basis of life history characters in a polychaete exhibiting planktotrophy and lecithotrophy. *Evolution* **45**(2):380-397.

McEdward, L. R. & L. K. Coulter. 1987. Egg volume and energetic content are not correlated among sibling offspring of starfish: implications for life-history theory. *Evolution* **41**(4):914-917.

Mileikovsky, S. A. 1971. Types of development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. *Mar. Biol.* **10**:193-213.

Palmer, A. R. & R. R. Strathmann. 1981. Scale of dispersal in varying environments and its implications for life histories of marine invertebrates. *Oecologia* **48**: 308-318.

Rumrill, S. S. 1986. Natural mortality of marine invertebrate larvae. *Ophelia* **32**(1-2):163-198.

Roff, D. 1992. The Evolution of Life Histories: Theory and Analysis. New York: Chapman and Hall. 535pp.

and the evolution of marine prosobranch gastropods. *In* Marine Organisms: Genetics, Ecology and Evolution. *Eds*. B. Battaglia and J. A. Beardmore. New York: Plenum Press: 303-322.

Sokal, R. R. & F. J. Rohlf. 1981. Biometry: the Principles and Practice of Statistics in Biological Research, 2nd ed. New York: W. H. Freeman. 859 pp.

Strathmann, R. R. 1985. Feeding and non-feeding larval development and life history evolution in marine invertebrates. *An. Rev. Ecol. Syst.* **16**:339-361.

_____. 1986. What controls the type of larval development? Summary statement for the evolution session. *Bull. Mar. Sci.* **39**(2): 616-622.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.* **25**:1-45.

Vance, R. R. 1973A. On reproductive strategies in marine benthic invertebrates. *Am. Nat.* **107**:339-352.

_____. 1973B. More on reproductive strategies in marine benthic invertebrates. *Am. Nat.* **107**:353-361.

Chapter 7

Poecilogony in *Haminaea*: General conclusion

The opisthobranch *Haminaea callidegenita* has variable development based on the dispersal potential of offspring. Both lecithotrophic veligers and juveniles hatch from every egg mass. Hatchlings pass through the same developmental stages at the same time, and the difference arises from the time of metamorphic competence. Half of the larvae per egg mass become competent inside the egg mass and are induced to metamorphose by a small, polar compound found in egg mass jelly. The other half hatch as veligers and subsequently become competent over a 14 d planktonic period. Time of competence is heritable and is little affected by culture conditions (both field and laboratory observations), parental reproductive traits, or time of spawning.

Time of competence after hatching is initially rapid, and then continues at low rates throughout the rest of the planktonic period. As a consequence, most swimming larvae have a short dispersal period that may result in their retention within or near the parental habitat. The number of "long range" dispersers rapidly decreases with time. This may be advantageous as the benefits of dispersal are thought to be reduced with increased advection from favorable habitats (Palmer & Strathmann 1981) or increased exposure to planktonic predators (Rumrill 1986). Throughout this period, hatched veligers will respond to an increasing variety of inductive substrates (although maintaining some discrimination) and will undergo "spontaneous" metamorphosis as well. This decrease in larval "choosiness" is important ecologically because it may allow larvae to locate a suitable habitat. The mechanism by which this may occur is not known but possibilities include late development of external receptor organs in addition to an organ receptive to the inducer found in egg mass jelly, or the activation of receptor sites in the original receptor organ.

This type of poecilogony is advantageous because every individual produces both swimming larvae and crawl-away juveniles; therefore each parent produces both dispersive propagules and recruits that may settle immediately in the parental population. This pattern may be advantageous in habitats that are regionally stable but locally ephemeral, such as seagrass beds. Two processes are thought to influence life history evolution in opisthobranchs: availability of food and stability of habitat (Clark 1978). If these two factors are continually available to a species, direct development is commonly observed; if they are unpredictable, patchy, or ephemeral, planktotrophy or planktonic development is common (Clark 1978; Hadfield & Miller 1987). Members of an existing *Haminaea callidegenita* population would have a constant supply of food. Food resources (diatoms

and detritus) are not likely to be limiting in seagrass beds because of the high productivity of this habitat (Zieman & Wetzel 1980); also, these resources are known to support a population of adults and juveniles throughout the year (Gibson & Chia 1991). However, seagrass beds change unpredictably from year to year. Freshwater run-off and winter storms rearrange the topography of these beds, either moving the drainage channels in which *H. callidegenita* concentrate at low tide (pers. obs.), or completely removing entire beds of grass. Winter conditions are occasionally severe and can cause adults to disappear, as has occurred in Spencer's Spit. In this population, *H. callidegenita* are known to be abundant and reproductively active year-round; in one unusually cold winter, animals disappeared only to re-appear in the late spring (Gibson & Chia 1991). Therefore, local conditions would favor retention of most offspring and the release of some dispersive veligers would counter the risk of local extinction.

How did this development pattern arise in *Haminaea callidegenita*? Possibly an ancestor produced lecithotrophic larvae that metamorphosed soon after hatching (within hours to days). Larvae that became competent earlier than their siblings may have metamorphosed within the egg mass. There may have been rapid selection for early metamorphosis if time of competence was as highly heritable as observed in the present population. Early metamorphosis would provide these offspring with the advantages of an early onset of juvenile feeding, faster growth, and earlier maturity. The development of swimming larvae would be set back chronologically but perhaps more importantly, would be set back energetically as well. These are non-feeding larvae that are unable to replenish energy reserves while planktonic. A late metamorphosis may have consequences similar to those known to occur in other species for competent larvae that delay metamorphosis (such as higher mortality, slower juvenile growth and delayed onset of maturity; Pechenik 1990). The retention of dispersive offspring in this life history would allow colonization of new populations, gene flow among existing populations, and perhaps short range dispersal from the parental locality but still within the Padilla Bay estuarine system.

Is poecilogony in *Haminaea callidegenita* evolutionarily stable or is it evolving towards direct development? Developmental and genetic data support the hypothesis that poecilogony in *H. callidegenita* may be a stable life history pattern. Developmentally, *H. callidegenita* veligers have a long swimming phase before they become competent to metamorphose (to 30 d). If poecilogony is evolving towards direct development, one might expect the dispersive phase to be much shorter. However, genetic correlations indicate that selection for intracapsular metamorphosis may increase the time to first competence of hatched larvae from the same clutch, at least in the short term. Therefore, the

proportion of direct developers may increase, but duration of the planktonic period would increase as well, preserving the poecilogonous life history.

Game theory also suggests that development patterns such as poecilogony may be an evolutionarily stable strategy (ESS; Maynard Smith 1982). ESS suggests that a genetic polymorphism may create a mixed strategy that is stable if the fitness payoff to an individual playing one strategy is the same as as the payoff resulting from the second strategy (Maynard Smith 1989). Therefore, a mixed development pattern involving the production of both dispersive and non-dispersive offspring may be the most successful development mode to a species living in a habitat that is not resource limited but may be seasonally unpredictable. This mode of development would involve spreading the risk, by combining immediate recruitment of juveniles to a "good" habitat with continual food and an existing population of prospective mates, with dispersal to habitats or existing populations potentially removed from the same environmental pressures.

Literature Cited

Clark, K. B. 1978. Nudibranch life cycles in the Northwest Atlantic and their relationship to the ecology of fouling communities. *Helgolander wiss. Meeresunters* **27**:28-69.

Gibson, G. D. and F. S. Chia. 1991. Contrasting reproductive modes in two sympatric species of *Haminaea* (Opisthobranchia: Cephalaspidea). *J. Moll. Stud.* **57**:49-60.

Hadfield, M. G. and S. E. Miller. 1987. On developmental patterns in opisthobranchs. *Am. Malac. Bull.* **5**(2):197-214.

Maynard Smith, J. 1982. Evolution and the Theory of Games. Cambridge: Cambridge University Press. 224 pp.

_____. 1989. Evolutionary Genetics. Oxford: Oxford University Press. 325 pp.

Palmer, A. R. and R. R. Strathmann. 1981. Scale of dispersal in varying environments and its implications for life histories of marine invertebrates. *Oecologia* **48**:308-318.

Pechenik, J. A. 1990. Delayed metamorphosis by larvae of benthic marine invertebrates: does it occur? Is there a price to pay? *Ophelia* **32**:63-94.

Rumrill, S. S. 1986. Natural mortality of marine invertebrate larvae. *Ophelia* **32**(1-2):163-198.

Zieman, J. C. and R. G. Wetzel. 1980. Productivity in seagrass: Methods and rates. *In.* Handbook of Seagrass Biology: An Ecosystem Approach. *Eds.*: R. C. Phillips and C. P. McRoy. New York: Garland STPM Press. pp. 87-116.